10-28-2016 12:00 AM

A Stable Isotope Investigation Of Pollen From Pinery Provincial Park, Southwestern Ontario, Canada

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Graduate Program in Geology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

Abstract

Pollen is a useful proxy for reconstructing paleoclimate; however, the relationship between its stable isotopic composition and the environment in which it forms is still poorly understood. We examine the stable oxygen, carbon, and nitrogen isotope compositions of pollen from tree (coniferous and deciduous), grass (C_3 and C_4), and marsh species from Pinery Provincial Park, Ontario, and its environs, and maple and oak tree species from eight localities across the United States of America, to (i) determine the major environmental influences on pollen $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ values, (ii) examine the causes of stable isotopic variability of pollen from different plant species from the same location, and (iii) assess whether the isotopic composition of pollen corresponds to that of plant cellulose.

Assuming a cellulose-water fractionation factor of +27‰, $\delta^{18}O$ values of *T. latifolia* (cattails) closely match $\delta^{18}O_{precipitation}$ values at the time of sugar synthesis in the area, suggesting little to no isotopic enrichment. Grass species differ from $\delta^{18}O_{precipitation}$ values at the time of sugar synthesis by +3.5 to +5.8‰, and tree species by +10.2 to +13.2‰, indicating increased levels of transpiration, and its attendant effects on $\delta^{18}O_{leaf\ water}$ values, prior to pollen synthesis. Bulk leaf cellulose $\delta^{18}O$ values differ by +0.6 to +2.1‰ relative to pollen in tree species versus up to +5.2‰ in grass species. These results suggest that the biochemical pathway used by sugars during translocation in grass species results in mixing of ^{18}O-poor source water with leaf water. The $\delta^{13}C$ value of pollen is likely controlled by a combination of factors such as photosynthetic pathway, and relative humidity (water stress) at the time of the formation of sugars used in pollen formation. The nitrogen isotope composition of pollen reflects a complex array of parameters, which were not resolved in this study, but likely reflects site-specific conditions affecting the more open versus more closed nature of the nitrogen cycle.

Keywords

Pollen, Oxygen Isotopes, Carbon Isotopes, Nitrogen Isotopes, Pinery Provincial Park, Cellulose, Vegetation.

Acknowledgments

Throughout my studies, I have received much support and encouragement from many wonderful individuals. I would like to thank my supervisor, Dr. Fred J. Longstaffe, for his continued guidance, support, and encouragement. In addition to teaching me the skills necessary to be a successful, independent thinker, he has also provided patience and stability throughout a sometimes difficult journey. I am also very grateful to Li Huang and Kimberly Law for their guidance and support in and out of the lab. To my thesis committee members Dr. Patricia Corcoran, and Dr. Elizabeth Webb, I thank you for your advice. Elizabeth, in particular for being a positive force when the dark side loomed heavy. I would also like to thank Grace Yao, for her endless support in the lab; Alistair MacKenzie at Pinery Provincial Park for allowing me to have the most beautiful study site in the world; Meghan Dammeier for her cherished friendship; Dr. Neil Banerjee, for his advice and a much-needed kick-in-the-pants, and the staff and faculty who helped facilitate my studies.

Pollen analysis for this study was possible only through the kindness of strangers. My sincere thanks go to Bob Byers, Dr. Mark Dixon, Monique Reed, Dr. Brett Serviss, Dr. Alexey Shipunov, Dr. Michael Stine, and Chris Watson. Your genuine eagerness to collect and send samples to a random student in Canada is forever appreciated.

This research was supported by a grant awarded to Dr. Fred. J. Longstaffe by the Natural Sciences and Engineering Council of Canada.

Finally, I would like to thank my family, friends, and colleagues for their support and encouragement throughout my studies. In particular, my parents, Reinhard and Terry, have been a source of constant inspiration and support. Thank you for teaching me to ask questions, and to always turn my failures into motivation to succeed.

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Chapter 1

1 Introduction

1.1 Overview

Plant tissue composition and molecular kinetics within a plant are commonly studied to better understand how vegetation responds to environmental influences, and this information in turn helps when reconstructing local and regional paleoenvironments. Using multiple methods of analysis such as tree rings (Leavitt and Long, 1991; Gulbranson and Ryberg, 2013) and faunal distribution (Börner et al., 2013), a more comprehensive understanding of ancient settings may be developed. Pollen grains are especially useful paleo-reindicators, because of their high preservation potential in the geologic record (Gandolfo et al., 1998; Sangster and Dale, 1961). More recently, there has been a focus on the stable isotopic composition of pollen, to determine its relationship with environmental parameters such as relative humidity and temperature. Amundson et al. (1997), for example, used the stable carbon isotope composition of pollen to identify C_3 versus C_4 ecosystems, and Loader and Hemming (2001) have shown that pollen $\delta^{13}C$ values are positively correlated to temperature.

The present study adds to this small but growing body of work by reporting the oxygen, carbon, and nitrogen isotope compositions of pollen of the tree species *Pinus resinosa*, *Pinus strobus*, and *Quercus rubra*, the grass species *Ammophila breviligulata* (C_3) and *Andropogon gerardii* (C_4), and the marsh species *Typha latifolia*, from Pinery Provincial Park (the Pinery), Ontario, Zurich, Ontario, and London, Ontario (Fig. 1.1). These locations share the same moderate temperate climate, altitude and weather patterns, and therefore are suitable locations for the study of pollen from different species. *Pinus resinosa* and *P. strobus* were selected to represent two different but abundant species of coniferous trees, and *Q. rubra* was selected as a deciduous tree species. Because grass pollen differs from tree pollen in formation timing and microclimate, two species (C_3 and C_4) were chosen for this study. Finally, *T. latifolia* was selected for analysis because it differs from both trees and grasses in that it grows in standing water.

This study also reports the isotopic compositions of pollen from *Quercus* species from Madison, FL, Monticello, FL, Arkadelphia, AR, Hot Springs, AR, College Station, TX,

Vermillion, SD, Minot, ND, and *Acer* species from Seattle, WA (Fig. 1.2). These locations were chosen based on their differences in temperature, relative humidity, altitude, and latitude. These data are used here to better understand the environmental controls that influence the isotopic composition of pollen and other plant tissue. To avoid influences in isotopic composition resulting from different genera, the same genus (*Quercus*) was selected from all sites, with the exception of Seattle, WA, where *Quercus* pollen was unavailable for analysis, and *Acer* pollen was analyzed instead.

1.2 Objectives of this thesis

The overall aim of this study is to determine how environmental parameters influence the oxygen, carbon, and nitrogen isotope compositions of pollen of different species of plants over four successive years. If present, relationships between the isotopic composition of pollen and climate variables could increase the potential of pollen as a reliable proxy for environmental conditions.

The research questions addressed in this thesis are:

(1) What are the major environmental influences affecting the oxygen, carbon, and nitrogen isotope composition of pollen? In this study, we evaluate factors such as temperature, relative humidity, altitude, and latitude.
(2) What factors control the variability in pollen isotope compositions among genera from the same location? Plants analyzed at the Pinery are exposed to the same overall weather systems, altitude, and latitude, which allows species-specific effects and/or differences resulting from microclimatic conditions to be evaluated.
(3) What factors control the variability in pollen isotopic compositions within species from the same location?
(4) Is the pollen isotope composition of plants examined in this study the same as the major tissue component, cellulose? If the isotopic compositions of both structures are similar, the isotopic analysis of pollen grains from the sediment record is a good proxy of the isotopic composition of the plant itself, and not just its pollen.

In order to address these research questions, this thesis will:

(1) Measure the oxygen, carbon, and nitrogen isotope compositions of pollen collected from C_3 and C_4 plants at and near the Pinery, over a four-year period, to determine how annual variations in environmental parameters affects the isotopic composition of pollen.

(2) Record daily relative humidity and temperature at the Pinery over the same four-year period, and determine if there is a relationship between these parameters and the isotopic composition of pollen.

(3) Measure the oxygen isotope composition of monthly precipitation for the Pinery over this four-year period, to determine if the oxygen isotope composition of pollen is dependent on the oxygen isotope composition of ambient precipitation.

(4) Measure the oxygen, carbon, and nitrogen isotope compositions of cellulose from plants for which pollen was analyzed and explain the similarities and differences between these two sets of results.

(5) Measure the oxygen, carbon, and nitrogen isotope compositions of pollen from various sites across the United States of America, to determine whether there are geographic or climatological variations on a continental scale that affect the isotopic composition of pollen, and if these variations differ from those observed at the Pinery.

1.3 Pollen as proxies for paleoenvironment

Studies of fossilized pollen grains provide valuable information about the living environment of the plant species that formed them. Conventional pollen environmental reconstruction relies on the collection of numerous pollen grains from the sediment record, and the identification of all plant species present based on the pollen types observed in the sample (Finkelstein et al., 2005; Trombold and Israde-Alcantara, 2005). Species assemblages identified from pollen in the sediment record can provide a good estimation of local

temperature and rainfall amounts. Such morphology-based methods are nonetheless subject to error in identification and classification of pollen grains, resulting from potentially similar-looking grain structures. In addition to classification errors, problems associated with conventional pollen studies include the time lag between environmental forcing and plant response (Loader and Hemming, 2000). Variations in the environment commonly result in physiological changes or adaptations in vegetation; however, these changes usually require a certain length of time to occur. As such, conventional pollen analyses commonly provide information on plant taxa adapted to past environmental conditions and are not necessarily reflective of the environmental conditions in which the grain was produced.

Stable isotope analysis of pollen grains is an alternative approach to palynologically based, paleoenvironmental reconstruction (Nelson et al, 2007; Loader and Hemming, 2001). In particular, photosynthetic processes and variations in atmospheric CO_2 levels are reflected in the stable carbon isotope composition ($\delta^{13}C$) of pollen grains, and as such, provide a measure of plant $\delta^{13}C$ values at the time of growth (Loader and Hemming, 2000). The $\delta^{13}C$ values of pollen grains found in the sediment record can therefore provide useful information on the timing and magnitude of past environmental changes. Recent advances in pollen isotope analysis require as little as a single pollen grain for each isotopic measurement (Nelson et al., 2007), thus ensuring that pollen from different years are not analyzed together.

The major component of the outer layer (exine) of pollen grains is sporopollenin. Because of its high sporopollenin content, exine is extremely resistant to chemical and mechanical degradation and is therefore abundant in the fossil record (Brooks and Shaw, 1978). Sporopollenin is a β-carotenoid ester, and has the approximate formula $C_{90}H_{150}O_{33}$ (Loader and Hemming, 2004). Stable isotope analyses can be performed on its carbon, hydrogen, and oxygen components, and used to inform paleoenvironmental interpretations. In particular, carbon isotope analyses of pollen grains help to determine if the plant species utilized a C_3 or C_4 photosynthetic pathway (Nelson et al., 2006). Modern C_3 plants have $\delta^{13}C$ values between –28 and –22‰ VPDB[1] and C_4 plants have $\delta^{13}C$ values between –16 and –10‰ VPDB (Descolas-Gros et al., 2001). The oxygen ($\delta^{18}O$) and hydrogen isotope ($\delta^{2}H$)

[1] Vienna Peedee belemnite (VPDB) standard (Coplen, 1994)

compositions of pollen grains may reflect the isotopic composition of local precipitation and may therefore provide useful information about past water compositions (Loader and Hemming, 2004). Nitrogen, however, present in pollen as proteins, has $\delta^{15}N_{plant\ tissue}$ values that are controlled by multiple factors such as temperature and precipitation amount, which can vary regionally based on latitude and altitude (Amundson et al., 2003), but also can vary significantly locally, depending on the nitrogen sources and the nature of the nitrogen cycle at any particular site (Tahmasebi, 2015).

Studies of the carbon isotope composition of pollen grains and their relation to environmental parameters are relatively few, but have increased in number over the past two decades (Amundson et al., 1997; Jahren, 2004; Nelson et al., 2007; Urban et al., 2010). By comparison, measurements of oxygen and nitrogen isotope compositions are rare, and their relationship to environmental parameters such as precipitation, relative humidity, and soil concentration is only poorly understood. This thesis aims to increase our level of understanding on the major controls on the oxygen isotope composition of pollen, strengthen existing interpretations of pollen $\delta^{13}C$ values and begin to explore possible factors affecting pollen $\delta^{15}N$ values.

1.4 Study area

As briefly noted earlier, this study focuses on pollen and plant vegetation collected from various plant species in the Pinery Provincial Park (Pinery), southwestern Ontario (Fig. 1.1), cattails collected in Zurich and London, Ontario, and oak (and maple) pollen from locations across the United States of America.

1.4.1 Pinery Provincial Park

Pinery Provincial Park is an ideal study location because it is a temperate Oak savannah forest, and has a large diversity of plant species as well as ecological niches, such as forested and dune environments. Because of this setting, the Pinery has been the site of many studies

and much information is available about the flora and fauna in this region. Additionally, the oxygen and hydrogen compositions of water (precipitation, surface water, groundwater) at the Pinery have been sampled over the past two decades by the University of Western Ontario (Longstaffe, unpublished data), which serves as a baseline for the isotopic composition of precipitation and groundwater during this time.

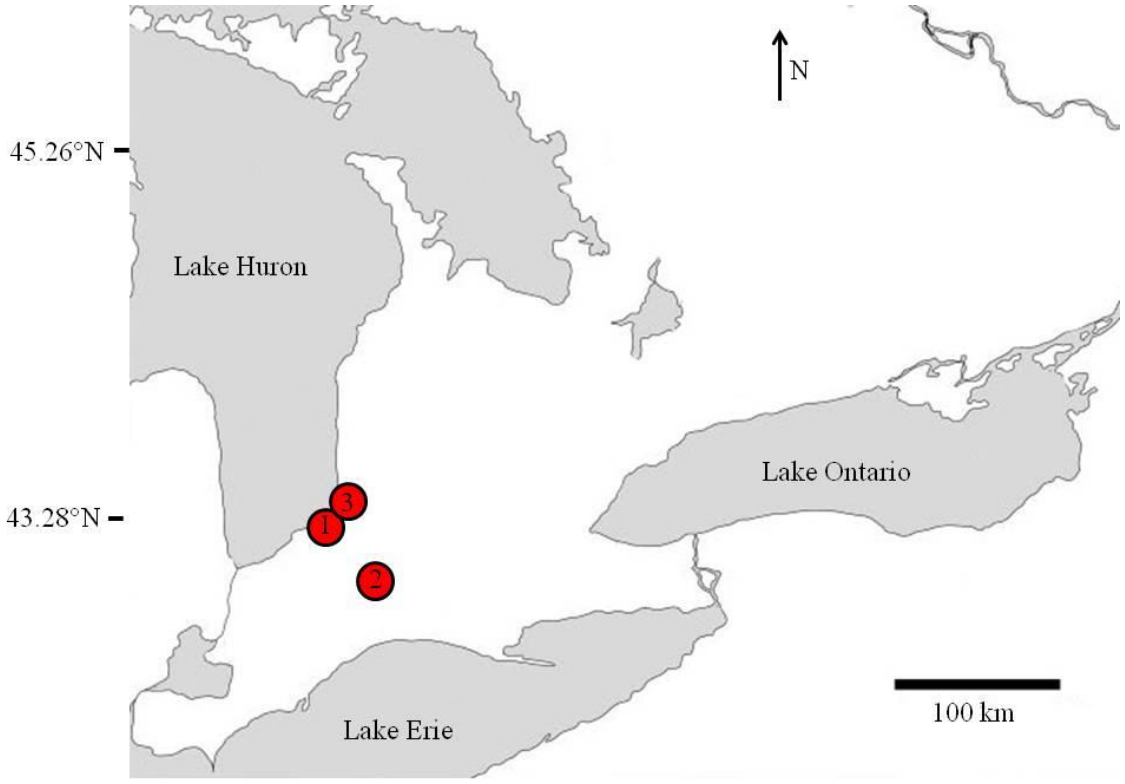

Figure 1.1 Map of southwestern Ontario, showing locations for (1) Pinery, Ontario, (2) London, Ontario, and (3) Zurich, Ontario.

The Pinery is located at 43˚ 15’ N, 81˚ 49’ W, along the southeastern shore of Lake Huron. It is part of the Great Lakes region and has a continental climate that is greatly influenced by the surrounding lakes (Hare and Thomas, 1979). The mean annual precipitation in the form

of rain or snow for this region ranges from 800 to 1000 mm and mean temperatures range from –20 to –10˚ C in winter, to 20 to 25˚ C in summer (Hare and Thomas, 1979).

The geology of the Great Lakes region has been severely modified by glacial activity during the last stadial and related changes in hydrological regime since that time. At the Pinery, a series of sand dunes mark the recession of Lake Huron and its precursors over the past 6,000 years. The youngest dunes, nearest the shore, are characterized by grasses and shrubs whereas older dunes are vegetated by an oak savannah ecosystem.

The Pinery is host to multiple habitat types. Its oak savannah habitat is home to over 70 species of trees and is maintained through prescribed burns and periodic pine cutting (Tagliavia et al., 2002). The harsh habitat of the freshwater coastal dunes is ideal for many species of shrubs, grasses, sedges, and insects (Morrison and Yarranton, 1973). Temperatures in these habitats show large variation, reaching summer (growing season) values above 40˚ C during the day and sometimes falling below 15˚ C at night. In addition to terrestrial habitats, the Pinery is also home to the 64 km-long Old Ausable Channel (OARC) – a slow-flowing river inhabited by several endangered freshwater mussels, fish and turtle species. Once part of the Ausable River, the OARC was isolated over the past 140 years after a series of channels were dug that diverted most of its water flow, and dams were developed on the OARC itself to enhance its potential as a recreational area.

The tree species analyzed in this study from the Pinery are *Pinus resinosa* (Red Pine), *Pinus strobus* (White Pine), and *Quercus alba* (White Oak). Grass species analyzed from this location are *Ammophila breviligulata* (C_3 grass), and *Andropogon gerardii* (C_4 grass). These species were selected because: (i) they are native to southwestern Ontario and in particular have a long history in the Pinery, and (ii) they are available in larger quantity than other tree and plant species, especially for the purpose of pollen collection.

1.4.2 Zurich, Ontario

Zurich, Ontario is located at 43˚ 25’ N, 81˚ 37’ W, just northwest of the Pinery (Fig. 1.1). Here, *Typha latifolia* (cattails) were collected from a drainage ditch beside a quiet road.

Overall climate patterns are similar to the Pinery; however, this small location has no canopy vegetation, and is exposed to full sunlight throughout the day. The drainage ditch in which the cattails grow remains filled with water throughout the growing season.

1.4.3 Locations across North America

Tree pollen samples were obtained from various locations across the United States of America (Fig. 1.2). Each location has its own set of unique climatic and environmental conditions, which are summarized in Table 1.1, along with climatic information obtained from the U.S. Climate Data Weather Service (http://w2.weather.gov/climate, 2015) and The Weather Network (http://www.theweathernetwork.com, 2015).

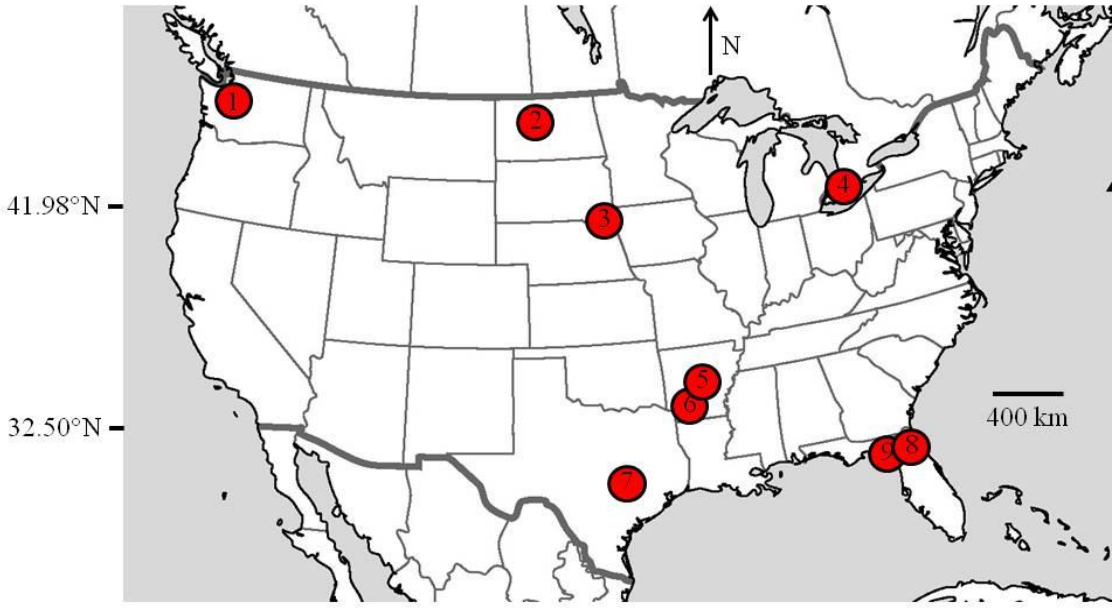

Figure 1.2 Map of North America showing all sampling sites: (1) Seattle, WA, (2) Minot, ND, (3) Vermillion, SD, (4) Pinery, Ontario, (5) Hot Springs, AR, (6) Arkadelphia, AR, (7) College Station, TX, (8) Madison, FL, and (9) Monticello, FL.

Table 1.1 Location and climate information of pollen collection sites across North America.

	Coordinates	**Species collected**	**Average high temperatures (˚C)**	**Average rainfall (mm)**
Pinery	43˚17’12.2424”N 81˚48’02.9700”W	*Q. rubra*	–2˚ (winter) 20˚ (summer)	1100-1200
Madison, FL	30˚28’32.8368”N 83˚25’22.5624”W	*Q. virginiana*	19˚ (winter) 33˚ (summer)	1300-1400
Monticello, FL	30˚30’58.3164”N 84˚2’13.0920”W	*Q. virginiana*	18˚ (winter) 32˚ (summer)	1400-1500
Arkadelphia, AR	34˚7’52.1364”N 93˚3’31.5324”W	*Q. shumardii*	13˚ (winter) 33˚ (summer)	1300-1400
Hot Springs, AR	34˚26’4.6068”N 93˚2’49.2792”W	*Q. stellata*	15˚ (winter) 29˚ (summer)	1400-1500
College Station, TX	30˚37’13.0692”N 96˚21’59.7564”W	*Q. stellata* *Q. comptoniae* *Q. shumardii* *Q. virginiana*	17˚ (winter) 35˚ (summer)	1000-1100
Vermillion, SD	42˚46’51.5604”N 96˚55’3.2124”W	*A. saccharium*	1˚ (winter) 29˚ (summer)	1500-1600
Minot, ND	48˚13’58.6812”N 101˚17’32.2476”W	*Q. macrocarpa*	–5˚ (winter) 26˚ (summer)	1600-1700
Seattle, WA	47˚36’44.7876”N 122˚20’14.8668”W	*A. macrophyllum*	8˚ (winter) 22˚ (summer)	900-1000

Chapter 2

2 Background

Studies investigating paleoclimate using fossil pollen assemblages have been in practice for over one hundred years. The diverse structures on the outer wall of pollen from different species provide a means to identify and categorize the plant species from which the pollen is derived. This chapter presents some background about the formation, morphology, and function of pollen, and describes previous research on stable isotope studies of pollen.

2.1 Pollen structure

2.1.1 Pollen morphology

The pollen grain of both angiosperms and gymnosperms contains the male gametophyte of the plant. Its function is to make contact with its female counterpart, the stigma (angiosperms) or the ovule (gymnosperms), to produce a seed. Although the overall shape and outer features of pollen grains are highly variable among species, pollen from all plants share a set of characteristic traits common to all species. The grain itself is generally composed of two main parts – the inner cell, and a pollen wall (Fig. 2.1). The pollen wall, which serves to protect the male reproductive material sheltered inside the grain, is further divided into a middle layer called the intine, and a resistant outer layer called the exine, which itself is further divided into morphological sections such as the ektexine (Jarzen and Nichols, 1996). The ektexine may have connected columnar structure (tectate), or it may be columnless or have unconnected columns (intectate).

The living portion of the grain is the inner cell, which houses the gametophyte necessary for stigma contact and reproduction. There are two nuclei inside the pollen grain: the generative nucleus and the vegetative nucleus (Fig. 2.2). The generative nucleus is eventually engulfed by the much larger vegetative nucleus, after which it produces two

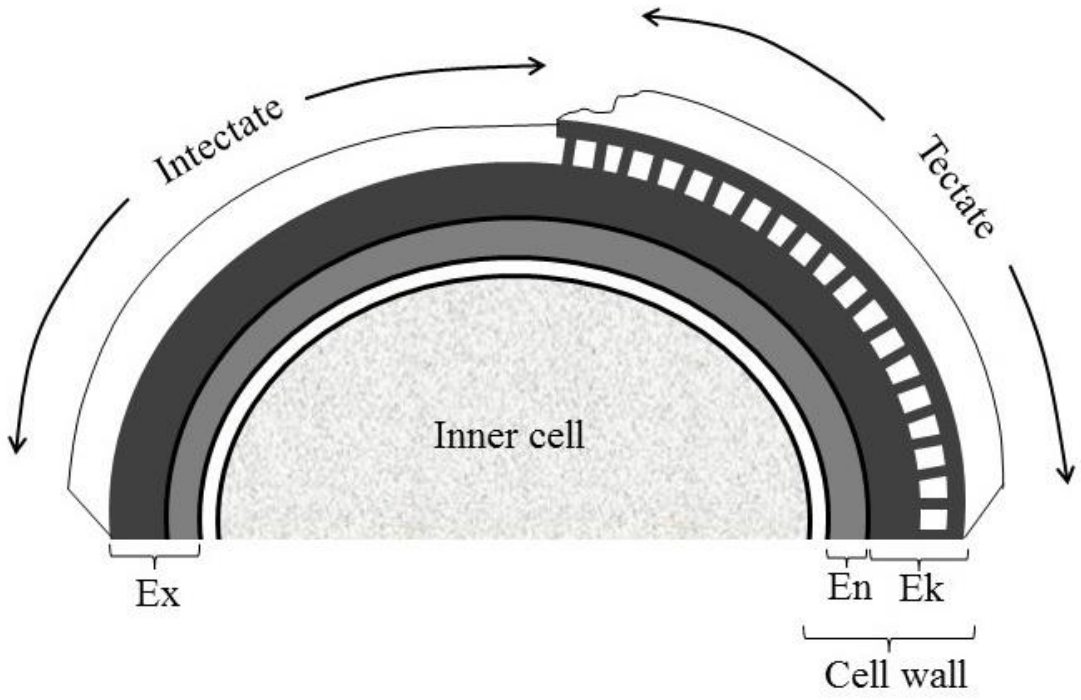

Figure 2.1 Schematic diagram of the layers of a pollen grain showing exine (Ex), endexine (En), and ektexine (Ek) in the cell wall, and the inner cell (Modified from Jarzen and Nichols, 1996).

male gamete (sperm) cells. The vegetative nucleus serves to transfer the sperm cells to the embryonic sac via an elongated pollen tube (Tanaka, 1997). Surrounding these cells is the vegetative cytoplasm, which is rich in water and proteins, and interrupted by numerous vacuoles; as nuclear development occurs, the cytoplasm becomes richer in lipid content (Kuang and Musgrave, 1996).

Lining the inner part of the pollen wall, the cell is uniformly surrounded by the intine (Fig. 2.3), which is composed mainly of cellulose $(C_6H_{10}O_5)_n$, a polysaccharide found in the cell walls of green plants (Fang et al., 2008). To a lesser extent, the intine is also composed of various proteins, and pectin, a heteropolysaccharide commonly found in cell

walls (Marquez et al., 1997). Unlike the outer wall layers, intine tends to be less stratified, and only slightly lamellar (Knox and Heslop-Harrison, 1970). This inner wall

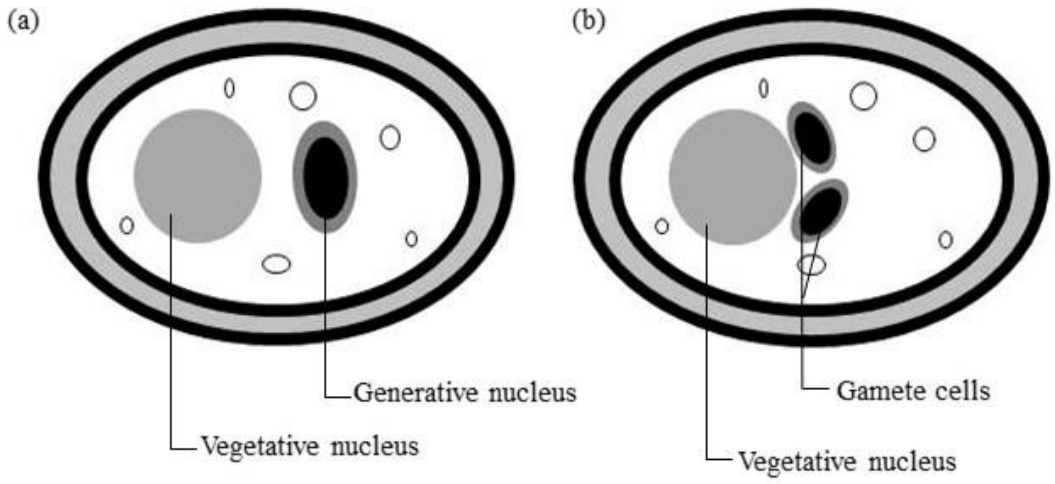

Figure 2.2 Schematic diagram of pollen grain showing cytoplasm, (a) vegetative nucleus and initial generative nucleus; and (b) vegetative nucleus and subsequent pair of gamete (sperm) cells.

layer is highly susceptible to degradation and therefore almost never undergoes fossilization (Faegri and Iversen, 1989).

Most plant species bear pollen grains that are covered in a tough outer layer called the exine (Fig. 2.3). This shell is highly resistant to decay because it is partially composed of a substance called sporopollenin. It is considered one of the most resistant organic substances in nature and remains intact long after the intine and central grain have decomposed (Faegri and Iversen, 1989). Sporopollenin is formed by the oxidative polymerization of carotene and carotene esters (Brooks and Shaw, 1968). The relative strength of sporopollenin is not correlated with its percentage present in the exine, but rather the degree to which it is polymerized, suggesting that pollen grains exhibit a range in quality with respect to exine resistivity (Faegri and Iversen, 1989). The amount of sporopollenin within the exine varies by as little as 3.5% in the genus *Phleum* to 24% in

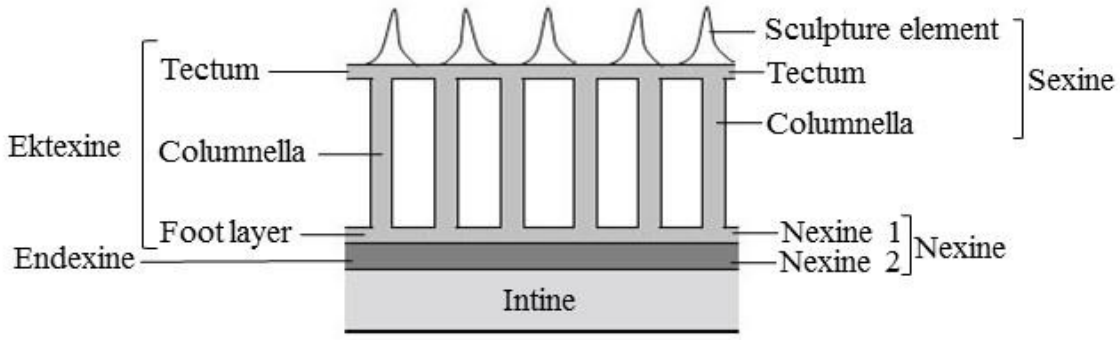

Figure 2.3 Schematic representation of the layers of a pollen grain showing the divisions of the exine and intine (Modified from Moore et al., 1991).

Pinus, and has a weak, positive correlation with cellulose content (Brooks and Shaw, 1973).

Traditionally, the exine is divided into the outer, highly sculptured sexine, and the inner, non-sculptured nexine (Fig. 2.3). The sexine is composed of radially arranged rods supporting a roof (tectum), and it overlies the bottom layer (nexine). The nexine may be divided into a top layer, commonly referred to as the foot layer to the sexine, and a bottom layer called the endexine, although these layers are often difficult to distinguish (Moore et al., 1991). Collectively, the sexine and the foot layer are called the ektexine. The entire exine resists degradation by concentrated acids and bases, and for short intervals can withstand temperatures of almost 300˚C (Faegri and Iversen, 1989).

2.1.2 Pollen diversity

Pollen grains exhibit a wide range of morphological variations and as such, these differences are used in species identification. In particular, it is the surface of the exine that is highly sculptured; however, the exact function of the different ornamental features

remains unclear (Moore et al., 1991). The purpose of the exine is to protect the inner, germinating nucleus of the grain from mechanical damage and desiccation.

The final shape and structure of a particular pollen grain may in part be a result of irregularities within the exine called apertures (Moore et al., 1991). Apertures are defined as any thin openings in the ektexine that do not conform to the regular pattern of the exine in which it is found. Apertures serve as the passageway of the pollen tube during germination, and as exit sites for proteins; therefore, it is likely that a larger number of apertures in the pollen grain provides a better chance for the pollen tube to reach the stigma, ensuring reproductive success. The presence of numerous apertures within a pollen grain suggests that their variation in number, size, and structure contributes to some extent to the overall morphology of the pollen grain itself. Another possible factor of exine variability with respect to morphology is the ability to which it can adhere to pollination vectors (Moore et al., 1991). Grayum (1986) states that pollen grains with long spines adhere readily to the hairs on insects such as flies whereas grains with smooth surfaces are better adapted to adhere to the similarly smooth surfaces of insects such as beetles.

Pollen grains also exhibit a wide range of diversity with respect to diameter, measuring from 5 to 200 μm (Faegri and Iversen, 1989). Although living pollen grains expand and contract in size depending on moisture content, exine shells found in sediments without their respective intine and cellular components are uniformly consistent in size, and this size varies significantly among species.

The identification and classification of fossilized pollen grains is largely based on the characteristics and number of apertures, which are divided into pores (pori) and furrows (colpi) (Moore et al., 1991). When identifying a particular grain, prefixes are added depending on the number of apertures present, e.g., mono-, di-, tri-, tetra-, penta-, and hexa- (Fig. 2.4). For example, a pollen grain with three pores would be triporate and a grain with four furrows would be tetracolpate (Jarzen and Nichols, 1996). Grains with more than six apertures are referred to as polyporate or polycolpate. If the apertures are

spaced equally around the equator of the grain, the prefix zono- is added and if the apertures cover the entire grain, the prefix panto- is included (Fig. 2.4).

2.1.2.1 *Pinus* pollen

The genus *Pinus* comprises all pine trees, which belong to the Family Pinaceae. Pine trees are evergreen, coniferous plants that are widely distributed globally, particularly in the northern hemisphere. In *Pinus*, male and female cones are present; the female cone, or ovulate cone, is woody and tends to be larger than the male cone. It contains ovules

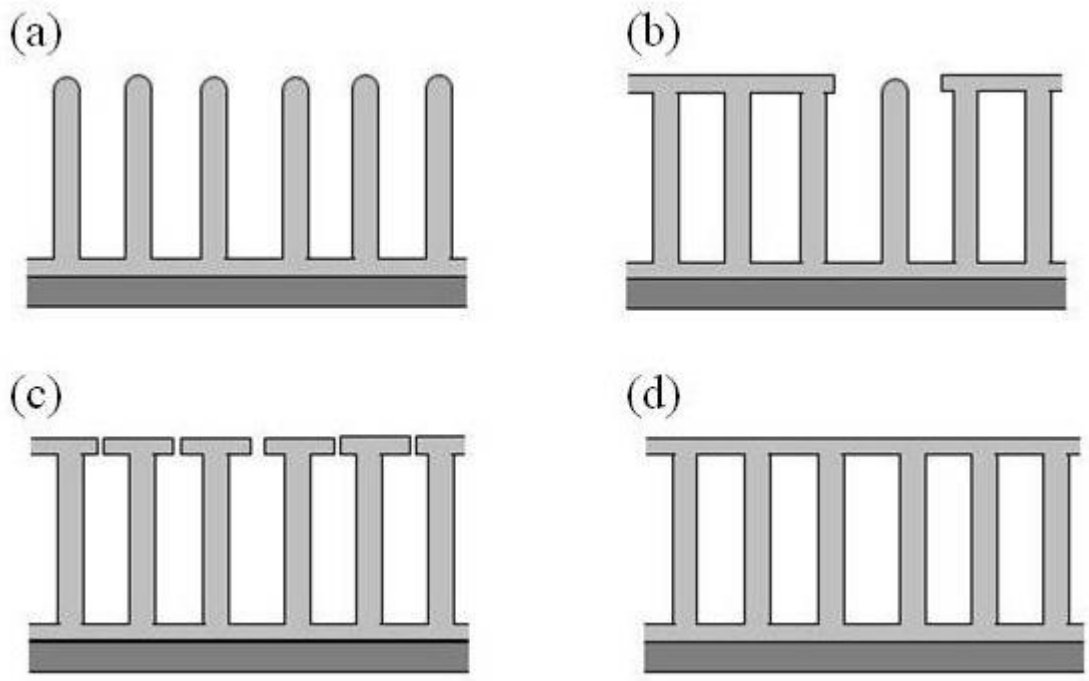

Figure 2.4 Schematic diagram of the divisions of the exine layer in angiosperms showing (a) columnless intectate structure; (b) partially developed tectate, called semi-tectate structure; (c) tectate perforate; and (d) completely developed tectate, called tectate imperforate (Modified from Jarzen and Nichols, 1996).

that produce seeds once they are fertilized by the pollen from a male cone. These cones are typically found in upper branches of the tree. Male cones, or staminate cones, tend to be smaller, and produce large amounts of pollen in mid-Spring (Fig. 2.5). These cones are generally found in the lower branches of pine trees, and are covered in modified leaves called scales (Fig. 2.5), each of which covers two pollen sacs. The release of pollen (pollen fly) is triggered by a combination of factors such as photoperiod, temperature, and most importantly, water supply (Clary et al., 2004; Eriksson, 1968). At the Pinery, pollen fly generally occurs in *Pinus* between the second to fourth week of May.

(a) (b)

Figure 2.5 Photograph of staminate cone of *Pinus resinosa* (Red Pine) (a) just before pollen fly in mid-May, and (b) during pollen fly, at the Pinery.

It is important that *Pinus* pollen be able to utilize wind currents for dispersal. Owing to the localized separation of male and female reproductive cones, pollen must travel upwards to reach the ovules contained in the female cones. As such, the pollen grain is morphologically adapted for maximum wind dispersal. It consists of a main body, with two laterally situated sacs, or bladders, which are responsible for carrying the pollen grain great distances or to great heights via wind currents. *Pinus* releases large amounts of pollen, and as such is often over-represented in sediment records (Li and Yao, 1990).

2.1.2.2 *Quercus* pollen

Quercus, the genus that comprises all oak trees, belongs to the beech Family Fagaceae, and is native to the northern hemisphere. Male and female flowers form separately, with male (staminate) flowers forming first, and female (pistillate) flowers forming directly after staminate production. Staminate flowers are produced in groups referred to as catkins (Fig. 2.6).

Germination is dependent on environmental factors such as photoperiod, temperature, and water supply. Staminate flower growth is completed approximately 1-2 weeks following the opening of leaf buds, and pollen is released 2-4 days after flowers are mature (Ducousso et al., 1993). Pistillate flowers, conversely, are much smaller, and form a few days subsequent to staminate production. They are located at the base of leaf stems, and have short stalks; they are commonly difficult to distinguish from emerging leaf buds (Ducousso et al., 1993). Following pollination of a female ovule, seed growth takes approximately 3 months, after which acorns are mature and fall to the ground. In the Pinery, *Quercus rubra* (Red Oak) typically releases pollen in mid-May, and acorn maturation is complete by late August.

The dispersal rate of the pollen grains of *Quercus* species is inversely proportional to their diameter; as such, the grains are relatively small (Solomon, 1983a). The distance pollen is able to travel increases when more staminate flowers populate higher regions of

the tree; when vegetation is thick, dispersal rates are negatively affected (Tauber, 1977). The grain is oval in shape, prolate, with short, straight furrows (Solomon, 1983a; Solomon, 1983b). *Quercus* pollen grains are present in moderate amounts in sediment records.

(a) (b)

Figure 2.6 Photograph of staminate flowers (catkins) of *Quercus rubra* (Red Oak) (a) just before pollen fly in mid-May, also showing female pistillate (white arrow), and (b) during pollen fly, at the Pinery.

2.1.2.3 *Ammophila* pollen

The C_3 genus *Ammophila* belongs to the Family Poaceae. Also known as Beachgrass, *Ammophila* is found extensively on coastal sand dunes, and is able to withstand high winds, arid conditions, and repetitive sand burial. Stems above ground often exceed 0.6 m in height, and have long, narrow leaves attached near the base of the stem. Its root system, comprising 30% of its biomass, is composed of an extensive network of rhizomes (Maun, 1984) that provide stability on windy shores, and allow the plant to access water at depth.

In *Ammophila* in southern Ontario, rhizome growth is initiated each April, and continues to grow until November (Krajnyk and Maun, 1980). Following initial rhizome growth in April, shoots begin to grow, after which secondary and tertiary rhizome growth occurs (Krajnyk and Maun, 1980). *Ammophila breviligulata* invests little energy into sexual reproduction; only a small percentage of plants produce flowers (Krajnyk and Maun, 1982). Originally hypothesized to be a result of non-viable pollen (Church, 1929), the low fertility rate of *A. breviligulata* is instead likely a result of low pollen yield and harsh environmental conditions (Krajnyk and Maun, 1982).

At the Pinery, *A. breviligulata* has been present for approximately 11,000 years; however, because of low abundances, its population increased in the early 1980s through human intervention in order to help stabilize the first string of dunes, which is located immediately adjacent to Lake Huron (Maun, 1985). *Ammophila breviligulata* is found solely on the windward side of this active dune, facing Lake Huron. Largely protected from human interference, *A. breviligulata* grows in dense populations. Pollen fly usually occurs from the last week of July to early August.

2.1.2.4 *Andropogon* pollen

Andropogon gerardii, a C_4 grass from the Family Poaceae, is tolerant of high winds, and is an ideal soil stabilizer. Similar to *A. breviligulata*, it is supported by an extensive rhizome network that may extend downwards by as much as 3 m. Above-ground stems can reach 2 m in height (Gustafson et al., 2004). Unlike *A. breviligulata*, *A. gerardii* invests more energy into pollen production; however, reproduction is likely limited by harsh environmental conditions (McKone et al., 1998).

At the Pinery, *A. gerardii* has a broader habitat range than *A. breviligulata*. It is found predominantly on the leeward side of the first and second dune complexes. Unlike *A. breviligulata*, which is the sole plant in its habitat, *A. gerardii* grows alongside many other grasses and trees, thereby competing for resources. Pollen fly occurs from the last week of July to the first week of August.

2.1.2.5 *Typha* pollen

Typha latifolia is a perennial marsh species, invasive to wetlands throughout North America. Stems reach between 1-3 m in height, with long, broad leaves attached at the bottom of the plant (Grace and Harrison, 1985). Roots form an extensive, fibrous subterranean system, where nutrients are stored for the energy necessary for spring growth. Inflorescences grow at the top of stems, and produce large amounts of wind-borne pollen. In Zurich and London, Ontario, pollen fly occurs from early to mid June.

2.1.3 Pollen formation

Pollen grains are formed by the meiotic divisions of a mother cell in the anther (or pollen sac), which produces a tetrad of four haploid microspores (Jarzen and Nichols, 1996). These four microspores are initially connected; however, only one develops into a mature

pollen grain. When they detach from each other, an inner layer of nutritive cells called the tapetum begins to form the outer wall (exine) (Moore et al., 1991). Pattern formation on the exterior surface of the grain occurs immediately following the isolation of each cell and the formation of a callose wall surrounding each grain. The callose layer, made of polysaccharides, separates pollen grains during development, and prevents the fusion of their exine walls (Nishikawa et al., 2005). Underneath this callose layer, a cellulose layer forms, which is punctuated by apertures and rods called probacula (Moore et al., 1991). These structures are composed of lipoprotein, and serve as support structures to the developing pollen grain (Moore et al., 1991). At this point, the nexine is deposited – the inner, unsculpted layer of the exine, and the callose wall begins to degrade to allow for the release of new pollen grains. Following maturation, pollen grains enter a phase of quiescence wherein together with the anthers they begin to dry out. It is only after pollen dispersal and pollination that they rehydrate and begin the process of germination.

2.2 Photosynthetic pathways

There are three photosynthetic pathways utilized by plants to convert atmospheric CO_2 into chemical energy for metabolic processes, C_3, C_4, and crassulacean acid metabolism (CAM). Each pathway is an adaptation to take advantage of a particular type of environmental setting. Plants that utilize the CAM method are predominantly succulents (e.g., cacti) and epiphytes (e.g., mosses); relative to plants that rely on the C_3 or C_4 pathways, their abundance is small and they are not considered further here.

2.2.1 C_3 photosynthetic pathway

C_3 plants thrive in temperate climates (Ehleringer et al., 1997). This photosynthetic pathway was the first to evolve, and is used by most plants. It is named as such because the first product of its photosynthetic cycle (Calvin Cycle) is a 3-carbon sugar phosphate called 3-phosphoglycerate ($C_3H_7O_7P$). In C_3 photosynthesis, atmospheric CO_2 enters the

leaf via small pores in the leaf called stomata. The CO_2 first combines with a 5-carbon sugar called ribulose bisphosphate (RuBP) with the help of the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco). This carboxylation process produces two 3-phosphoglycerate molecules (Fig. 2.7). Reduction of these molecules via the energy molecule adenosine triphosphate (ATP) produces the sugars and starches necessary for the metabolic functions of the plant. The enzyme Rubisco also uses oxygen, O_2, to catalyze a reaction that produces one molecule of phosphoglycerate and one molecule of phosphoglycolate, a 2-carbon molecule. This process, known as photorespiration, results in a reduction in overall carbon fixation and the formation of CO_2 (Ehleringer and Cerling, 2002).

At fixed CO_2 levels, increases in average growing temperature decrease the percentage of C_3 growth relative to C_4; at fixed temperatures, increases in CO_2 levels increase C_3 vegetative growth relative to C_4 (Still et al., 2003).

2.2.2 C_4 photosynthetic pathway

The C_4 photosynthetic pathway evolved independently at least 45 times in 19 families as an adaptation to the C_3 pathway (Sage, 2004). In order to take advantage of ecological niches subjected to high light intensities, temperatures, and aridity, C_4 plants evolved an additional step in their chemical breakdown of CO_2, resulting in a process less wasteful of CO_2 and oxygen (Gowick and Westhoff, 2011).

Upon entering the leaf, and before entering the interior sheath cells, CO_2 is modified in the surrounding mesophyll cells. Here, the enzyme phosphoenolpyruvate (PEP) carboxylase fixes the CO_2 into oxaloacetate, a 4-carbon acid (Fig. 2.8) (Ehleringer and Cerling, 2002). This 4-carbon acid then enters the interior sheath cell through diffusion, undergoes decarboxylation, and is then used in the same Calvin Cycle as in C_3 plants (Fig. 2.8). The additional step involving PEP carboxylase increases the concentration of CO_2 in areas where Rubisco is located, and results in a high ratio of CO_2 relative to O_2

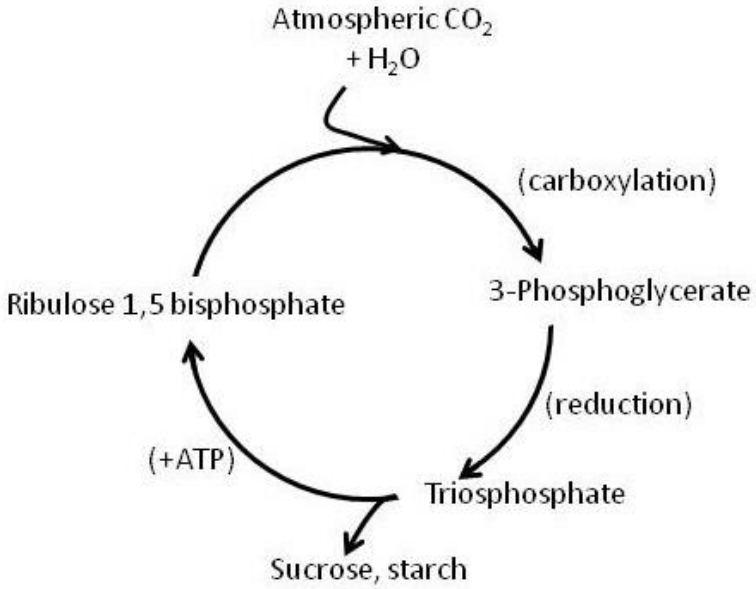

Figure 2.7 C_3 photosynthetic pathway showing the Calvin Cycle (modified from Ehleringer and Cerling, 2002).

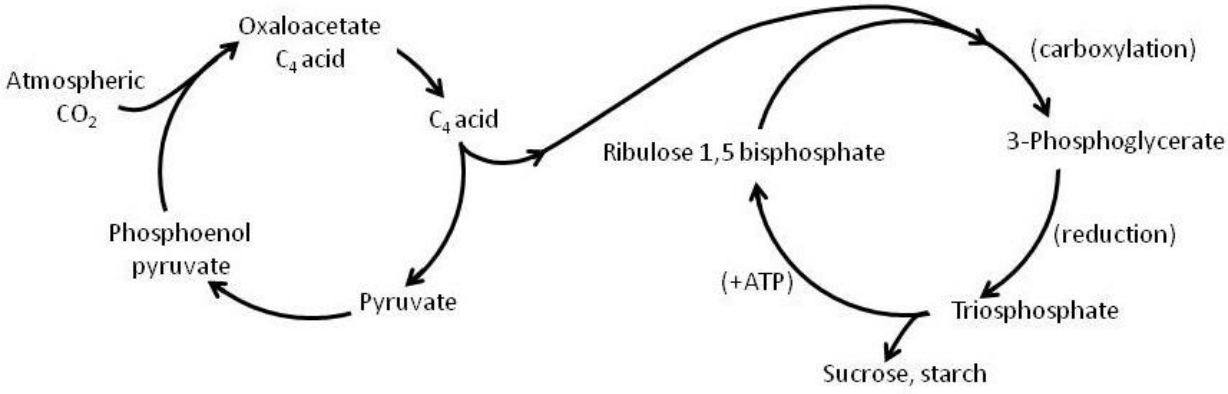

Figure 2.8 C_4 photosynthetic pathway showing Calvin Cycle and additional CO_2 modification step (modified from Ehleringer and Cerling, 2002).

(Ehleringer and Cerling, 2002). As such, C_4 plants do not undergo photorespiration; this energy-saving adaptation permits these plants to grow larger and faster than competing C_3 plants in the same area under the same conditions (West-Eberhard et al., 2011).

Plants that have evolved the C_4 photosynthetic pathway are found in regions with high temperature, low CO_2 levels, and/or high aridity. They are therefore commonly the dominant vegetative type in grasslands, and at low latitudes (Gowik and Westhoff, 2011). In Canada, native C_4 plants are mostly restricted to grasses that experience heat and dryness.

2.3 Carbon isotope composition of pollen grains

The carbon isotope composition of pollen has been investigated for at least the last two decades (Nelson, 2012; Jahren, 2004; Amundson et al., 1997). Carbon isotope analyses aid in the understanding of numerous processes such as localized atmospheric CO_2 and CO_2 processing (Buchmann et al., 2002; Marino and McElroy, 1991), and are used to categorizing photosynthetic pathways. The carbon source for plant tissue material is atmospheric CO_2, which enters the plant via stomata in the leaf epidermis. Carbon is then processed through carbon fixation and converted into the starches and sugars necessary for cell metabolism and tissue formation. At each step, carbon isotopes are fractionated; however, the degree to which they are fractionated is dependent on which photosynthetic pathway is used by the plant.

2.3.1 Carbon isotope variation among pollen of different plant species

The carbon isotope composition of pollen grains provides useful information about the ratio of C_3 versus C_4 plants present in a given paleoenvironment. The $\delta^{13}C$ values for C_3 plants generally range from –35 to –26‰; the average for most plants is approximately

–28‰ (Cerling, 1999; O'Leary, 1988). Conversely, for C_4 plants the average $\delta^{13}C$ value ranges from –14 to –12‰ (Cerling, 1999; O'Leary, 1988).

In C_3 plants, carbon isotopes are first fractionated when atmospheric CO_2 diffuses across the epidermis of the leaf via small pores called stomata. Here, ^{13}C is discriminated against, because it has a slightly higher mass, thus it moves more slowly than ^{12}C across the plant membrane, and because it also forms stronger chemical bonds, making it less accessible than ^{12}C (O'Leary, 1988). This results in an approximate –4.4‰ change in $\delta^{13}C$ values (Farquhar et al., 1989; Craig, 1954). Following the diffusion of CO_2 through the leaf epidermis, carbon is further fractionated during carbon fixation, in the carboxylation process. Here, the $^{13}C/^{12}C$ ratio in C_3 plants is fractionated by approximately –25 to –24‰ (Farquhar et al., 1989), though this value can vary slightly depending on the rate of diffusion of CO_2 across the stomata (O'Leary, 1988).

In C_4 plants, carbon isotopes are fractionated similarly to C_3 plants when CO_2 diffuses across the epidermis via stomata; however, because the carbon fixation process differs, the net change in $\delta^{13}C$ values is different in C_4 plants. Here, the first enzyme to fix carbon is phosphoenolpyruvate (PEP) carboxylase, which does not discriminate against ^{13}C to the same degree as in the carbon fixation cycle in C_3 plants (Farquhar et al., 1989). Carbon that is produced from the initial C_4 carbon fixation process then moves into the bundle sheath cells within the leaf, and ^{13}C is discriminated against by the enzyme ribulose bisphosphate carboxylase (Keeley, 1991). Using PEP carboxylation, the amount of enzyme carbon isotope fractionation is –5.7 to –5.4‰ relative to the CO_2 pool of carbon (Farquhar et al., 1989; Reibach and Benedict, 1977). Coupled with the fractionation of carbon isotopes during the diffusion of CO_2 through the stomata, the total fractionation between air and tissue for most C_4 plants is –12‰ (Cerling, 1999).

Because the $\delta^{13}C$ values of C_3 versus C_4 plants differ by approximately 15‰, it is possible to distinguish between these types of plants by analyzing the carbon isotope composition of pollen grains (Amundson et al., 1997; Loader and Hemming, 2000). Nelson et al. (2007) analyzed single pollen grains from both modern C_3 and C_4 grass species, and obtained average results of –26.9‰ (±6.3‰) and –11.5‰ (±9.6‰)

respectively. This shows that techniques employed to analyze the small quantities of pollen grains from the paleorecord can produce reliable $\delta^{13}C$ values. These $\delta^{13}C$ values show significant variation; in a study of 46 different families of C_3 plants, Jahren (2004) reported a range from –32.3‰ in pollen from *Pinus taeda* (Loblolly pine), of the Family Pinaceae, to –22.9‰ in pollen from *Osmanthus fragrans* (Sweet osmanthus), of the Family Oleaceae.

Timing of pollen formation may also influence its carbon isotope composition. In a study of *Pinus sylvestris* trees, Loader and Hemming (2004) report that the $\delta^{13}C$ values of pollen reflect the carbon isotope composition expected for the 4-6 week period prior to pollen fly. This strongly suggests that the carbon isotope composition of pollen depends on environmental conditions during time of formation, and species pollinating earlier in the season will differ from those pollinating later in the season.

2.3.2 Carbon isotope variation among pollen of the same plant species

The carbon isotope composition of pollen from the same plant species is also subject to variation. Jahren (2004) reports up to a 2.3‰ difference in $\delta^{13}C$ values in pollen of *Staphylea trifolia* (American Bladderwort), and Loader and Hemming (2001) report a difference in $\delta^{13}C$ values of up to 6.5‰ in pollen of *Pinus sylvestris* (Scots Pine).

Environmental factors such as temperature may influence the $\delta^{13}C$ values within species. Loader and Hemming (2001) report a strong positive linear relationship between the $\delta^{13}C$ values of pollen and ambient temperature during the developmental period of the pollen grains. This increase in $\delta^{13}C$ values with increasing ambient temperature during developmental period is attributed to an increase in carboxylation fractionation in the photosynthetic process, resulting from an increase in utilization of intercellular CO_2 relative to atmospheric CO_2. A rise in intercellular CO_2 may result from decreasing stomatal conductance or increased assimilation (Loader and Hemming, 2001). Jahren

(2004), however, reports a less consistent relationship between pollen $\delta^{13}C$ values and ambient temperature during developmental period. Only five of nine tree species showed an increase in pollen $\delta^{13}C$ values with increasing temperature during development, whereas the other four showed a decrease in pollen $\delta^{13}C$ values. It is possible that a positive correlation between temperature during development and the $\delta^{13}C$ values of pollen is species-dependent, and other factors may influence the carbon isotope composition of pollen in these species to a greater extent than temperature.

Local environment may also affect the carbon isotope composition of pollen, because it has been found to affect other plant tissues. *Quercus* (oak) leaves exposed to higher average sunlight amounts were found to have lower bulk tissue $\delta^{13}C$ values compared to leaves exposed to lower amounts of sunlight (Lockheart et al., 1997). Lockheart et al. (1997) attribute this negative correlation to changes in photosynthetic assimilation and stomatal response that occur with changes in light availability. Graham et al. (2014) similarly report that leaf $\delta^{13}C$ values from C_3 trees collected from the more northerly-situated Maryland, USA (less sunlight), were higher than C_3 leaf $\delta^{13}C$ values collected from a forest in Panama (more sunlight). In contrast, Pearcy and Pfitsch (1991) report higher $\delta^{13}C$ values in bulk leaf tissue of adjacent plants exposed to more, rather than less, sunlight, at constant growing heights. They attributed this difference in $\delta^{13}C$ values to the recycling of respired CO_2 within the forest canopy.

The canopy effect may have a larger effect on the carbon isotope composition of pollen from vegetation in forested areas. Bulk leaf $\delta^{13}C$ values from the well-aerated, sunlit upper canopy may be up to 10‰ more enriched in ^{13}C than bulk leaf tissue collected near or at the bottom of the forest floor (Graham et al., 2014; Van der Merwe and Medina, 1989). This vertical difference in carbon isotope composition is likely a result of CO_2 recycling under the forest canopy, which produces ^{13}C-depleted CO_2. On the forest floor, decomposing vegetative material produces ^{13}C-depleted CO_2, which is then incorporated into leaf tissue via photosynthesis (Van der Merwe and Medina, 1991). In addition to rotting biomass, an increase in forest density results in an increase in above-ground roots (air roots), particularly in Amazonian forests. These roots may produce up to 82% of the

total CO_2 respired from the ground, thus contributing to ^{13}C-depleted CO_2 in the lower gradients of forest canopies (Medina and Minchin, 1980). Farquhar et al. (1982), however, suggest that the lower $\delta^{13}C$ values observed with decreasing height in the forest canopy is a result of CO_2 availability in intercellular spaces in the leaf (C_i) relative to CO_2 in the air (C_a). Analyses of the carbon isotope composition of leaf material in dense Amazonian forests by Van der Merwe and Medina (1991) suggest that $\delta^{13}C$ values are influenced to some degree by both CO_2 recycling, and light availability.

Geographical location can also affect pollen $\delta^{13}C$ values, because it influences the $\delta^{13}C$ values of bulk leaf tissue in plants of the same species. In general, at similar atmospheric pressures, plant tissues grown at higher latitudes have higher $\delta^{13}C$ values than at lower latitudes (Körner et al., 1991). This trend coincides with an increase in the amount of ^{13}C in atmospheric CO_2 moving from tropical vegetative areas such as dense forest to arctic areas such as tundra (Lancaster, 1990). The carbon isotope composition of atmospheric CO_2 increases by as much as 5‰ along this latitudinal gradient, likely a result of CO_2 recycling within forested canopies. Similarly, $\delta^{13}C$ values increase with altitude (Hultine and Marshall, 2000; Körner et al., 1988); $\delta^{13}C$ values of leaves collected in lowland areas were as much as 4.8‰ lower than those at higher latitudes (Körner et al., 1988). Körner et al. (1988) attribute this relationship to the partial pressure of CO_2 within and external to the leaf. At higher altitudes, the partial pressure of CO_2 within the leaf is lower than the partial pressure of CO_2 in the air, thus limiting the amount of carbon available for carboxylation during photosynthesis, and decreasing ^{13}C discrimination.

Sampling location on an individual tree can also influence $\delta^{13}C$ values. As with the canopy effect, $\delta^{13}C$ values of bulk leaf tissue collected near the bottom of the tree are lower than those from the top of the tree (Berry et al., 1997). As explained for the canopy effect, this decrease in $\delta^{13}C$ values with lower height is attributed to less sunlight, and to the assimilation of CO_2, recycled through plant decay and/or respiration at or near the forest floor.

Water supply may also affect the $\delta^{13}C$ values of leaf tissues. Meinzer et al. (1990) report higher carbon isotope discrimination in expanding coffee plant leaves subjected to

regular irrigation compared to those exposed to drought-like conditions, whereas mature leaves under the same conditions did not yield a similar relationship. Zhang et al. (1997) report no significant relationship between $\delta^{13}C$ values and water supply in adolescent to mature *Pinus ponderosa* (Ponderosa pine) plants; however, well-watered seedlings yielded significantly lower $\delta^{13}C$ values (up to 2‰ lower) than seedlings that were water-stressed. To adapt to situations of periodic water stress, plants may increase their water-use efficiency in order to survive drought-like conditions. The carbon isotope composition of plant tissue is strongly positively correlated to increasing water-use efficiency (De Souza et al., 2005; Sun et al., 1996; Martin and Thorstenson, 1988; Farquhar and Richards, 1984). Water use efficiency increases in plants when stomatal apertures are reduced in size; this allows for less water loss out of the stomatal opening, but also permits less CO_2 from entering the leaf, thus lowering C_i (Bradford et al., 1983). Reduced C_i results in less ^{13}C discrimination by Rubisco, the carboxylating enzyme involved in the first step of carbon fixation, thus increasing $\delta^{13}C$ values of bulk leaf tissue.

2.3.3 Carbon isotope variation among plant tissues

Pollen is abundant in ancient sediments dating back to the evolution of seed plants, which were established by the Carboniferous. Spore-like morphological precursors to the pollen grain are found in sediments dating as far back as the Devonian (Gerrienne et al., 2004). Pollen is generally the only part of the plant preserved in the ancient sediment record, and therefore it is important to understand how the carbon isotope composition of pollen is related to various other tissues of the plant, and the different processes that may affect their $\delta^{13}C$ values.

Polysaccharides are the most common material in leaf and stem tissue, and comprise approximately 65-90% of the leaf and stem by mass (Jahren, 2004). Of these polysaccharides, cellulose $(C_6H_{10}O_5)_n$ is the most common, and contributes to the overall structural integrity of the plant. Relative to bulk tissue, cellulose commonly has $\delta^{13}C$

values that are ~ 1-2‰ higher (Benner et al., 1987); some studies report ^{13}C enrichment of up to 4‰ (Jahren, 2004). Rossmann et al. (1991) also found that within glucose, certain carbon atom positions have $\delta^{13}C$ values up to 10‰ higher than other positions in the structure.

Lignin, the non-sugar component of plant cell walls, is a complex polymer composed of phenylpropane units. Comprising approximately 9-30% of leaf tissue by mass (Jahren, 2004), the carbon isotope composition of lignin is lower than bulk tissue by ~ 4.2‰ in C_3 leaf samples and ~ 6.7‰ in C_4 leaf samples (Benner et al., 1987). Lignin measured in C_3 wood samples are slightly less depleted of ^{13}C relative to bulk tissue. Benner et al. (1987) report 1.5 to 3.3‰ depletion of lignin ^{13}C relative to bulk tissue; Loader et al. (2003) reported a depletion of 3‰ relative to bulk tissue. The depletion of ^{13}C is thought to result from fractionation during the synthesis of phenylalanine and tyrosine, molecules that are necessary for lignin production (Benner et al., 1987). Tyrosine is depleted of ^{13}C relative to many other amino acids, and its relative abundance in certain plant species may affect the $\delta^{13}C$ value of their lignin.

Lipids comprise ~ 1-5% (by mass) of stem and leaf tissue (Jahren, 2004), and their carbon isotope composition is affected by fractionation during photosynthesis. Although $\delta^{13}C$ values may vary among plant species, in general their lipids are one of the most ^{13}C-depleted of plant tissue components (Chikaraishi et al., 2003). In lipid biomolecules isolated from leaves of the Japanese cedar tree *Cryptomeria japonica*, Chikaraishi et al. (2003) report a ^{13}C depletion of 2.4 to 9.9‰ relative to bulk tissue. This ^{13}C depletion occurs when glucose is metabolized to form the lipid molecule (DeNiro and Epstein, 1977). After glucose is first converted to pyruvate, the pyruvate undergoes decarboxylation and oxidation by pyruvate dehydrogenase to form acetyl coenzyme A (CoA), which is then used in the production of the lipid molecule. This decarboxylation and oxidation is responsible for the large fractionation in ^{13}C atoms that are then used in lipid biosynthesis (DeNiro and Epstein, 1977).

The carbon isotope composition of pollen has a strong positive correlation to bulk tissue values (Nelson, 2012; Jahren, 2004). Nelson (2012) reports a mean increase in $\delta^{13}C$

values of ~ 1.4‰ for pollen relative to bulk leaf tissue values, and a mean $\delta^{13}C$ increase of ~ 0.5‰ for isolated sporopollenin relative to bulk leaf tissue values.

In addition to differences in the average $\delta^{13}C$ values of various plant tissues, location within the plant is also a factor in determining carbon isotope composition. Jahren (2004) reports a 0.1‰ mean decrease in $\delta^{13}C$ values of leaf cellulose relative to stem cellulose of the same plant, in a broad sampling of C_3 plants. Leavitt and Long (1982) report a much larger decrease of 2 to 4‰ of leaf cellulose versus stem cellulose in Juniper trees. The carbon isotope composition of bulk leaf tissue is consistently lower than the carbon isotope composition of pollen and stem material; however, pollen and stem $\delta^{13}C$ values differ between plant types. In trees with predominantly woody stems, the $\delta^{13}C$ values of pollen are lower than the $\delta^{13}C$ values of stem material, whereas in trees with predominantly green, more flexible stems, the $\delta^{13}C$ values of stem material is lower than that of pollen (Jahren, 2004).

2.4 C/N ratios of pollen grains

The C:N (carbon to nitrogen ratio) measures the ratio of the mass of carbon to the mass of nitrogen in a sample, and can vary between plant groups and different parts of the same plant. At the broadest scale, aquatic algae tend to have C:N ratios between 4 – 10, whereas terrestrial plants have higher ratios (Ishiwatari and Uzaki, 1987). Among terrestrial sediments, coniferous trees yield C:N values >20, whereas deciduous trees yield C:N values between 9 – 12 (Descolas-Gros and Schölzel, 2007). Consistent with this, Descolas-Gros and Schölzel (2007) report average C:N values of pollen in coniferous trees of 37, and in deciduous trees 9. This large difference in C:N values is a result of differences in nitrogen rather than carbon (Descolas-Gros and Schölzel, 2007).

2.5 Oxygen isotope composition of pollen grains

Measurements of the oxygen isotope composition of pollen are not as commonly made as those of carbon isotope composition; however, pollen oxygen isotope compositions may be a useful tool for evaluating paleoenvironmental conditions. Loader and Hemming (2004) report a strong negative relationship between the oxygen isotope composition of pollen grains and the $\delta^{18}O$ values of precipitation. This pattern contrasts with the positive relationship between the $\delta^{18}O$ values of precipitation and the $\delta^{18}O$ values of tree rings reported in many studies (Danis et al., 2006; Rebetez et al., 2003; Saurer et al., 1997), and the corresponding negative relationship between the $\delta^{18}O$ values of precipitation and amount of rainfall (Xu et al., 2013). Loader and Hemming (2004) suggest that the negative relationship between the $\delta^{18}O$ values of pollen and the $\delta^{18}O$ values of precipitation may reflect the use of stored sugars in the production of pollen grains. They speculate that because leaves are either not yet formed, or have not reached optimum photosynthetic rates early in the growing season, pollen grains, which form at this time, do not use sugars produced at the same time. Instead, pollen must utilize stored photosynthates formed at an earlier time, such as the previous growing season; however, comparisons of the oxygen isotopic composition of pollen to environmental parameters of previous growth periods was not included in their study.

Chesson et al. (2013) report no significant relationship between the $\delta^{18}O$ values of pollen and the $\delta^{18}O$ values of precipitation, and speculate that this may be because plant water is largely influenced by microclimate such as temperature and relative humidity, which can heavily alter the oxygen isotope composition of the water immediately surrounding the plant. Such microclimate changes significantly throughout the growing season, and among locations. The oxygen isotope composition of pollen, and its relationship to the oxygen isotope composition of precipitation, is still very poorly understood. It is unclear if the processes that affect the $\delta^{18}O$ values of pollen are analogous in any way to those of other plant tissues.

2.5.1 Oxygen isotope composition of leaf water and cellulose

Water taken up by the plant through the roots does not undergo isotopic fractionation; it does, however, affect the oxygen isotope composition of leaf water. Transpiration, the process wherein water travels from the roots to the leaf stomata and is subsequently evaporated into the atmosphere, greatly alters the $\delta^{18}O$ value of leaf water (Flanagan and Ehleringer, 1991). During transpiration, ^{16}O-rich water molecules are preferentially evaporated from the leaf, resulting in leaf water that is more enriched in ^{18}O. Evaporative processes during leaf transpiration approximate the equation for ocean water evaporation given by Craig and Gordon (1965); this equation, modified by Dongmann et al. (1974), describes leaf evaporation as follows:

$$\delta^{18}O_E = \delta^{18}O_S + \varepsilon^* + \varepsilon_k + e_a/e_i\,(\delta^{18}O_A - \varepsilon_k - \delta^{18}O_S) \qquad \text{(Equation 1.1)}$$

where $\delta^{18}O_E$ is the isotopic composition of the evaporative pool of the water undergoing transpiration in the leaf; $\delta^{18}O_S$ is the isotopic composition of the stem water; ε^* represents equilibrium isotopic effects; ε_k represents kinetic isotopic effects; e_a/e_i is the ratio of the ambient vapor pressure to the vapor pressure in the leaf; and $\delta^{18}O_A$ is the isotopic composition of atmospheric vapor. This equation illustrates the importance of the isotopic compositions of stem water, atmospheric water vapor, and the leaf-air vapor pressure gradient in controlling the overall isotopic composition of leaf water (Flanagan et al., 1991).

In both C_3 and C_4 plants, the oxygen isotope composition of leaf water varies considerably throughout the day (Dongmann et al., 1974). A similar discrimination against ^2H is observed on a daily basis (Leaney et al., 1985); for example, high temperatures combined with low relative humidity result in much higher $\delta^{18}O$ values than

low temperatures coupled with higher relative humidity (Leaney et al., 1985). Following this gradient, Leaney et al. (1985) report a much higher enrichment in δ^2H values for C_4 plants compared to C_3 plants. Flanagan et al. (1991), however, report no such differences in δ^2H and δ^{18}O values of leaf water between C_3 and C_4 plants. They attribute any isotopic differences in leaf water to possible species-specific or individual plant-specific morphological differences, that result in variable path lengths for water movement from unfractionated source water veins (e.g., xylem tissue water) to the sites of water evaporation (e.g., stomata), where isotopic fractionation is high.

Although the Craig-Gordon equation is commonly used to generally determine the δ^{18}O value of leaf water, it often overestimates the degree of isotopic enrichment (Wang et al., 1998). Because deviations from this model are common, it is difficult to precisely predict the δ^{18}O value of leaf water at any given time. It is generally accepted that the oxygen isotope composition of leaf water may be a continuum of values resulting from the contribution of environmental δ^{18}O values, physiological features, and differences in stomatal conductance during water loss (Barbour and Farquhar, 2000).

The isotopic composition of leaf water is related to that of other plant tissues such as cellulose (Epstein et al., 1977) and phytoliths (Webb and Longstaffe, 2000). As such, a sound understanding of the processes that affect leaf water are important when studying the isotopic composition of other plant tissues.

2.5.2 Oxygen isotope composition of leaf cellulose

This study explores the relationship between the oxygen isotope composition of pollen and cellulose, and how water affects their final δ^{18}O value. The oxygen isotope composition of leaf cellulose is dependent on the oxygen isotope composition of leaf water (Barbour et al., 2000). Because changes in the δ^{18}O value of leaf water translate to changes in the δ^{18}O value of leaf cellulose (Helliker and Ehleringer, 2002), measuring the δ^{18}O values of leaf cellulose may offer clues to environmental conditions during cellulose

synthesis. When cellulose, a polysaccharide with the general formula $(C_6H_{10}O_5)_n$, is formed, oxygen isotope fractionation occurs in a single exchange step, during carbonyl hydration (Sternberg, 2012; Yakir and DeNiro, 1990). The formation of cellulose from its glycerol precursor results in an ^{18}O-enrichment of ~ 27‰ relative to ambient leaf water.

Studies evaluating the reliability of the $\delta^{18}O$ value of cellulose as a proxy for environmental parameters suggest that it retains its environmental isotopic signature (Royles et al., 2012); however, various processes affect the degree of reliability. Events such as high rainfall or very high relative humidity may force the oxygen isotopic signal of cellulose to be significantly different from that of its source water, and thus non-representative of environmental conditions (Royles et al., 2012; Helliker and Griffiths, 2007).

The oxygen isotope composition of leaf water, and thus leaf cellulose, differs depending on which photosynthetic pathway is utilized by the plant. Helliker and Ehleringer (2000) observed ~ 4‰ enrichment in leaf water in C_4 plants versus C_3 plants. Later work supports this C_4 enrichment; however, as relative humidity increases, the disparity in $\delta^{18}O$ values in leaf water between C_3 and C_4 plants becomes smaller (Helliker and Ehleringer, 2002).

2.5.3 Oxygen isotope composition of tree ring cellulose

Because cellulose from tree rings is the most commonly studied plant tissue analyzed for oxygen isotope composition (Young et al., 2015; Treydte et al., 2014; Sternberg, 2009), its reliability as a proxy to environmental parameters must be established. The $\delta^{18}O$ values of tree ring cellulose exhibit a strong correlation with temperature (Libby et al. 1976) and relative humidity (Burk and Stuiver, 1981). DeNiro and Cooper (1990), however, argue that tree ring cellulose may not be an accurate indicator of specific environmental parameters such as relative humidity, and Terwilliger and DeNiro (1995)

report that relative humidity is not recorded in tree ring cellulose. Van der Sleen et al. (2015) also report no significant relationship between the oxygen isotope composition of tree ring material and that of precipitation, although factors such as poor geographical proximity of measured tree material to precipitation $\delta^{18}O$ recording stations may contribute to this lack of correlation.

Most evidence strongly suggests that the oxygen isotopic composition of tree ring cellulose is dependent on the isotopic composition of leaf water, and thus reflects isotopic changes that occur in leaf water. The changes in the $\delta^{18}O$ value of water as it travels to the trunk to form tree-rings are complicated. Unfractionated source water first travels to the leaf where it undergoes fractionation related to transpiration. Carbohydrates formed in the leaf are then transported to a tissue layer between the xylem and the phloem called the cambium, which serves as an active growing site for the stem or root of the tree.

Much debate surrounds the amount to which interaction with source water influences the $\delta^{18}O$ value of mobile carbohydrates. In the potato plant, for example, the oxygen isotope composition of carbohydrates is completely over-written by incoming source water, thus suggesting that cellulose analysis is a reliable indicator of source water only (Terwilliger and DeNiro, 1995). Roden and Ehleringer (1999) argue that this is not representative of trees composed of cambium, as the storage of, and processes affecting, the carbohydrates involved in cellulose production differ considerably from that of non-tree species. Gessler et al. (2009) report that the oxygen isotope signal of leaf water was predictably transferred to cellulose in tree rings in *Pinus sylvestris*, with the expected isotopic enrichment of 27‰; they also report 40% exchange between organic oxygen and xylem (source) water oxygen prior to cellulose synthesis.

The $\delta^{18}O$ values of tree ring cellulose has long been used as an indicator of ambient temperature during its formation; more recent studies suggest that this relationship has more contributing factors that must be considered in paleoenvironmental reconstruction from such data (Royles et al., 2012). The oxygen isotopic composition of cellulose is

affected by factors such as leaf temperature (Helliker and Ritcher, 2008), and leaf-to-air vapor pressure differences (Kahmen et al., 2011).

2.6 Nitrogen isotope composition

2.6.1 Nitrogen isotope composition of pollen grains

Few studies have analyzed the nitrogen isotope composition of pollen grains, which is present primarily in pollen proteins. Descolas-Gros and Schölzel (2007) report average $\delta^{15}N$ values of pollen ranging from +2.4 to +7.0‰ for deciduous trees, +1.4 to +4.0‰ for evergreen trees, and +2.4 to +4.2‰ for herbaceous plants, excluding outliers. In their study, $\delta^{15}N$ values of pollen from a small sample of C_4 plants range from +6.1 to +7.6‰. The $\delta^{15}N$ values of pollen are interpreted to be representative of the nitrogen isotope composition of soil, combined with the different mechanisms used by plants to assimilate and metabolize nitrogen (Descolas-Gros and Schölzel, 2007).

2.6.2 Nitrogen isotopes in soil and plant matter

There are various ways in which plants access nitrogen. Organic matter in the soil undergoes ammonification by microbial activity to produce ammonium (NH_4^+). These ions are then absorbed by the plant or further oxidized by nitrifying bacteria into nitrites (NO_2^-) and nitrates (NO_3^-), which can then be assimilated into the plant. Plants inhabiting soils with little organic matter may rely to some extent on atmospheric nitrogen.

The $\delta^{15}N$ value of soil is influenced by the amount of ^{15}N fractionation resulting from bacterial processes. These processes are a large part of the nitrogen cycle; they are responsible for reactions such as nitrification and ammonification, which produce large changes in soil $\delta^{15}N$ values (Robinson, 2001). In many plant species, bacteria living symbiotically on the roots convert nitrogen into a more accessible form for the plant.

Ammonification, an important step in the nitrogen cycle, is the process wherein the organic nitrogen of decaying organisms or animal waste in the soil is converted into ammonium ions (NH_4^+) or ammonia (NH_3) by microorganisms in the soil. In this process, bacteria break down the proteins and amino acids containing the nitrogen. Ammonium is more depleted of ^{15}N because glutamine synthetase, the enzyme used in its assimilation, is highly discriminatory – up to –16.5‰ relative to its source (Handley et al., 1996).

The next step in the nitrogen cycle is nitrification, which occurs in two steps, nitritation and nitratation (Schmidt, 1982). The first step, nitritation, occurs when ammonia-oxidizing bacteria oxidize ammonium ions or ammonia into nitrite (NO_2^-), with a net reaction of:

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O \qquad \text{(Equation 1.6)}$$

The conversion of NH_4^+ or NH_3 to nitrite is associated with a large isotopic fractionation of ^{15}N (Yoshida, 1998). In a study on the chemolithotroph bacterium *Nitrosomonas europaea*, Mariotti et al. (1981) report a nitrification enrichment factor during nitrification in a pure culture of –35‰. The next step in nitrification is nitratation, wherein nitrite is further oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria, with a net reaction of:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad \text{(Equation 1.7)}$$

This second nitratation step is not rate-limited, and therefore should not contribute any isotopic fractionation effects (Högberg, 1997).

Soil profiles also yield general trends in δ^{15}N values; progression down a vertical soil profile yields increasing δ^{15}N values (Vervaet et al., 2002). Vervaet et al. (2002) report an increase of 11‰ in δ^{15}N values in a deciduous forest floor soil profile. As roots take in nitrogen from the soil, isotopic fractionation occurs; the amount of fractionation, however, varies among studies (Högberg, 1997). Studies indicate a δ^{15}N fractionation factor resulting from nitrogen uptake from the soil of approximately –0.3 to –4.0‰ (Mariotti et al., 1980; Kohl and Shearer, 1980).

Overall, the nitrogen amount and isotopic composition of soil varies considerably geographically, and on the micro-environmental scale (Högberg, 1997). In particular, the laterally extensive root system of trees results in a potentially variable source of δ^{15}N soil values, complicating the prediction of the δ^{15}N values of tissues of different trees. The nitrogen isotope composition of vegetative matter may also differ with fluctuations in relative humidity (Handley et al., 1996).

The nitrogen isotope composition of plant matter depends largely on the amount of organic matter present in soil (Craine et al., 2015). Plants that rely exclusively on N_2 fixation have δ^{15}N values close to 0‰ – the nitrogen isotope composition of the atmosphere (Craine et al., 2015). This value may increase depending on the degree to which the plant depends on the atmosphere as a source of nitrogen.

Chapter 3

3 Material and Methods

3.1 Pollen sampling

The pollen of two coniferous tree species, *Pinus resinosa* (Red Pine), and *Pinus strobus* (White Pine), the deciduous tree species, *Quercus rubra* (Red Oak), the C_3 grass species *Ammophila breviligulata* (Beach grass), and the C_4 grass *Andropogon gerardii* (Big Bluestem grass), was collected at Pinery Provincial Park (Pinery), Ontario (see Chapter 1, Fig. 1.1). Pollen from *Typha latifolia* (cattails) was collected close to the Pinery at Zurich, Ontario, and in London, Ontario (see Chapter 1, Fig. 1.1). Pollen samples were also obtained from oak and maple trees at eight sites across the United States of America (see Chapter 1, Fig. 1.2).

The tree species studied are the most abundant at the Pinery. Most trees in this mature and densely-packed forest are too tall to allow for easy pollen collection, which limited sampling access relative to forest size. Only trees with viable low-hanging branches provided the opportunity for efficient pollen collection. These trees were located mainly adjacent to small, gravel parking lots or within small open glades within the forest, where sunlight reaches the bottom of the tree, thus allowing viable leaf and needle development on lower branches.

Pollen was collected from *P. resinosa* and *P. strobus* by first shaking the male pine cone (Fig. 3.1a, b) and catching the pollen in a clean, plastic bag. Under a microscope, all debris was then removed using tweezers, and the pollen air-dried prior to isotopic analysis. Like the conifers, *Q. rubra* pollen (Fig. 3.1c) was easily accessible only from trees with low-hanging branches that had access to sunlight (i.e., adjacent to small parking lots and in open glades within the forest). To collect *Q. rubra* pollen, inflorescences (flowers) were removed from the tree and air-dried. Once dry, the inflorescence was shaken over weighing paper, on which the pollen was collected, and then cleaned of debris under a microscope prior to isotopic analysis.

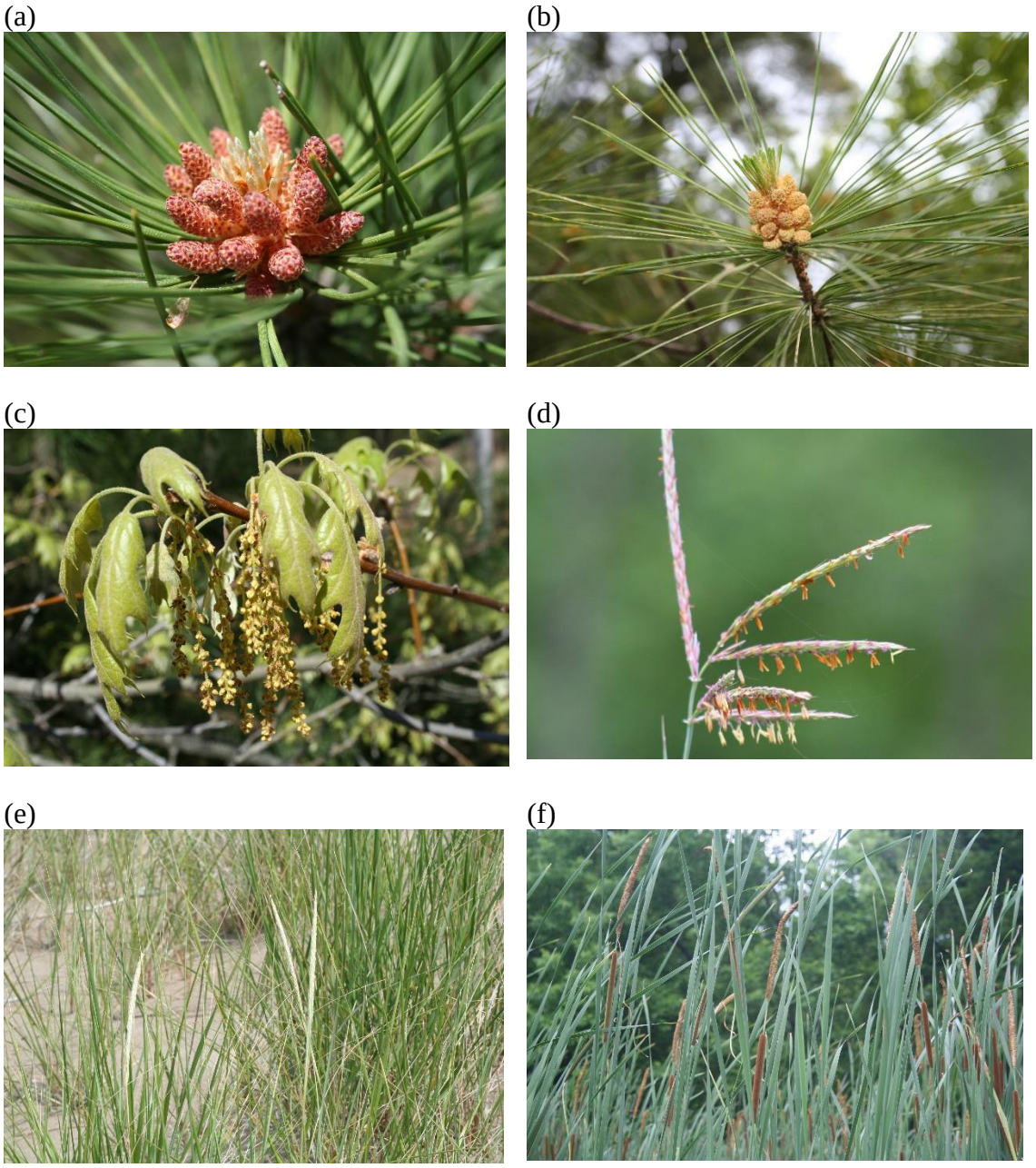

Figure 3.1 Photographs of: (a) male, pollen-bearing pine cone of *P. resinosa*; (b) male, pollen-bearing pine cone of *P. strobus*; (c) pollen-bearing inflorescences of *Q. rubra*; (d) *A. gerardii* flower and anthers; (e) *A. breviligulata* grass and flowers; and (f) pollen heads above flower heads of *T. latifolia*.

The C_4 grass *A. gerardii* (Fig. 3.1d) was collected in the same general locations as most tree species, on the leeward side of the second dune from the Lake Huron shoreline, in close proximity to a small parking lot. Grass here receives significant sunlight, but overhead vegetation provides shade in late afternoon. *Andropogon gerardii* was sampled just prior to pollen fly. Stems, leaves, and inflorescences were collected and thoroughly dried. Anthers were removed from spikelets, individually cut open under a microscope, and pollen extracted using a small pick. This was a labourious process, as a majority of dissected anthers had either lost their pollen already in the field, or had insufficient pollen for collection. Pollen was then inspected for purity under a microscope, and dried prior to isotope analysis.

The C_3 grass *A. breviligulata* (Fig. 3.1e) was collected on the windward side of the first dune, facing Lake Huron. Grass at this location is exposed to full sunlight throughout the day, has no overhead vegetation, and grows directly in the sand dune with little soil accumulation. Grass stalks with full, pollen-bearing inflorescences were chosen, and stems, leaves and inflorescences were collected. Similar to *A. gerardii*, anthers were isolated from spikelets, cut open under a microscope, and pollen removed prior to inspection, drying, and analysis.

In Zurich, Ontario, a large cluster of *T. latifolia* plants (Fig. 3.1f) growing in a drainage ditch alongside a quiet road was selected for pollen collection. In London, Ontario, an even larger area of *T. latifolia* growing in a marsh near a small forested area on the campus of The University of Western Ontario was selected for pollen collection. Pollen was collected by inserting the male staminate flower (located above the brown pistillate flower) into a small bag, and shaking the flower; each staminate flower produces large amounts of pollen. Pollen was then cleaned of debris under a microscope, and dried prior to analysis.

Pollen from the eight sites across the United States of America was collected by local researchers. Correspondence was sent to numerous research facilities across the United States, and a small number of those contacted agreed to collect and send tree pollen for analysis. Instructions for tree pollen collection, as described above, was sent to each

American colleague. Once received, the pollen was cleaned and dried prior to analysis. Pollen was collected from oak and maple trees that were readily accessible, as summarized in Chapter 1 (Table 1.1).

3.2 Sampling and preparation of material for cellulose analysis

For all tree, grass, and cattail species, leaves and stems were sampled for isotopic analysis of cellulose in 2011 and 2012 at the same time as pollen collection. Needles of *P. resinosa* and *P. strobus*, and leaves of *Q. rubra*, *A. breviligulata*, *A. gerardii*, and *T. latifolia* were collected and air-dried. The green stems attached to leaves of *Q. rubra* were detached and dried, as were the woody stems of *P. resinosa* and *P. strobus* attached to needles. Stems of *A. breviligulata*, *A. gerardii*, and *T. latifolia* were also collected and air-dried. Following drying, leaf and stem material was crushed into a fine powder, using a mortar and pestle. Using the method described by Brendel et al. (2000) to extract cellulose, acetic acid and nitric acid were added to the finely ground sample in a test tube, and heated at 120˚C for 30 minutes to separate cellulose. Ethanol was then added, and test tubes were centrifuged to isolate cellulose. Samples were rinsed three times by adding ethanol and acetone, centrifuging, and then decanting the liquid. Samples were then freeze-dried in preparation for isotopic analysis.

3.3 Sampling and preparation of plant water

Plant water was collected from leaves, needles, and stems of trees and grasses in 2011 and 2012. For each species, an individual tree or grass stalk was selected for plant water analysis based on how typical its local environment was compared to those routinely sampled for pollen and other plant parts. Leaves, needles, green stems (*Q. rubra*), and woody stems (*P. resinosa* and *P. strobus*) were cut from the tree as close to the source of

pollen collection as possible. Samples were inserted into glass sampling tubes, sealed, and immediately placed on ice until they were returned to the laboratory, where they were stored frozen until analysis.

To extract the water from the leaf, needle, and stem samples, glass tubes were filled (approximately one-quarter volume) with plant tissue and then sealed with a rubber stopper (after Leaney et al., 1985). The tubes were then attached to a needle fixed to a gas transfer apparatus. Water was extracted from samples under a vacuum for fifty minutes, while being heated at 100˚C.

3.4 Isotopic analysis

Oxygen, carbon, and nitrogen isotope results are expressed relative to a standard, using the δ-notation

$$\delta X = [(R_{sample}/R_{standard}) - 1] \text{ (‰)}$$

where X denotes ^{18}O, ^{13}C, or ^{15}N, and R represents the ratio between the heavy and the light isotopes ($^{18}O/^{16}O$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$). All isotopic measurements were performed at the Laboratory for Stable Isotope Science (LSIS), at The University of Western Ontario, London, Ontario.

3.4.1 Oxygen isotope measurements

Oxygen isotope results for pollen and cellulose were calibrated and reported relative to Vienna Standard Mean Ocean Water (VSMOW) (Coplen, 1994). Isotopic compositions were measured using a High Temperature Conversion Elemental Analyzer (TC/EA) peripheral attached to a dual-inlet triple-collecting, isotope-ratio mass spectrometer (IRMS). Samples were air-dried, after which 0.2-0.3 mg was measured into a silver

capsule, crimped, and then loaded into an auto-sampler. International standards IAEA-CH-6 (accepted value = +36.40‰) and Ennadai (accepted value = +22.20‰) were used to calibrate the oxygen isotopic values to VSMOW. Two internal laboratory standards, IAEA-CH-3 and Whatman cellulose, were used to evaluate the accuracy and precision (measured to ±1 standard deviation) of δ^{18}O values. The average δ^{18}O value of IAEA-CH-3 (accepted value = +31.30 to +32.70‰) was +32.5 ± 0.5‰ (n=25), and the average δ^{18}O value of Whatman cellulose (accepted value = +27.80‰) was +28.0 ± 0.2‰ (n=22). The reproducibility of δ^{18}O for the calibration standards was normally better than ±0.3‰ (n=55), and for duplicate samples was normally better than ±0.2‰ (n=21 pairs).

The oxygen isotope compositions of leaf, needle, and stem waters were analyzed using a Picarro L1102-I isotope liquid water and water vapor analyzer at the University of Western Ontario, and are reported relative to VSMOW. Laboratory standards LSD (accepted value = –22.57‰), MID (accepted value = –13.08‰), EDT (accepted value = –7.27‰), and Heaven (accepted value = –0.27‰), which are calibrated to the VSMOW=SLAP scale, were placed at the beginning, middle, and end of each analytical session. LSD (–22.6 ± 0.1‰, n=6) and Heaven (–0.3 ± 0.1‰, n=12) were used to calibrate the δ^{18}O values to VSMOW. MID and EDT were used to evaluate accuracy and precision, and compared well with accepted values and expected precision (MID: –13.0 ± 0.1‰, n=6; EDT: –7.3 ± 0.1‰, n=18). The reproducibility of δ^{18}O values for samples was normally better than ±0.1‰ (n=10 pairs).

3.4.2 Carbon and nitrogen isotope measurements

All samples were air-dried prior to isotopic analysis. Carbon and nitrogen isotopes were analyzed separately because the small amount of nitrogen present in samples relative to carbon required different sample weights. For carbon, 0.1-0.2 mg of sample was measured into tin cups and crimped, and for nitrogen, 3.2-3.5 mg of sample was measured into tin cups, then crimped. Carbon and nitrogen isotope compositions, and bulk elemental carbon and nitrogen compositions were measured using a Costech

Elemental Analyzer (EA) peripheral, attached to the Thermo Finnigan Delta^{plus} XL IRMS. Carbon isotope compositions were calibrated and expressed relative to Vienna Pee Dee Belemnite (VPDB) (Coplen, 1994), and nitrogen isotope compositions relative to air (AIR) (Böhlke and Coplen, 1993).

For carbon, the international standards USGS-40 (accepted value = –26.24‰) and USGS-41 (accepted value = +37.76‰) were used to calibrate samples to VPDB (Qi et al., 2003) and two standards, one internal (Keratin, Laboratory for Stable Isotope Science, The University of Western Ontario) and one international (IAEA-CH-6) were used to evaluate accuracy and precision (measured to ±1 standard deviation). The average δ^{13}C value of Keratin (accepted value = –24.04‰) was –24.1 ± 0.1‰ (n=31), and the average δ^{13}C value of IAEA-CH-6 (accepted value = –10.45‰) was –10.6 ±0.3‰ VPDB (n=22). The reproducibility of δ^{13}C for the calibration standards was normally better than ±0.3‰ (n=53), and for duplicate samples was normally better than ±0.1‰ (n=15 pairs). The accepted carbon content of keratin (48.2 ±1.1 wt.%) is comparable to the average obtained in this study (48.1 ±3.1 wt.%; n=31).

Nitrogen isotope compositions were calibrated to AIR using international standards IAEA-N-1 (accepted value = +0.4‰), IAEA-N-2 (accepted value = +20.3‰) (Böhlke and Coplen, 1993), USGS-40 (accepted value = –4.52‰), and USGS-41 (accepted value = +47.57‰) (Qi et al., 2003). Accuracy and precision (measured to ±1 standard deviation) were assessed using the internal standard Keratin. The average δ^{15}N value of Keratin (accepted value = +6.36‰ AIR) was +6.3‰ ± 0.2‰ (n=26). The reproducibility of δ^{15}N for the calibration standards was normally better than ±0.2‰ (n=26), and for duplicate samples was normally better than ± 0.2‰ (n=15 pairs). The accepted nitrogen content of keratin (14.8 ±0.7 wt.%) is comparable to the average obtained in this study (14.8 ±0.7 wt.%; n=26).

3.5 Statistical analysis

Descriptive statistical tests were performed using IBM SPSS statistics software (2015 version). One-way ANOVA was used to test for significant differences in the isotopic composition of pollen among years, among species, and among different locations. Tukey tests and Dunnett's T3 tests were subsequently used to identify which sets of data differed from each other in their isotopic compositions. Variances were considered significantly different at $P < 0.05$.

Chapter 4

4 Results

Oxygen, carbon, and nitrogen isotope results were obtained for three species of trees: *Pinus resinosa* (Red Pine), *Pinus strobus* (White Pine), and *Quercus rubra* (Red Oak); the marsh plant *Typha latifolia* (Cattails); the C_3 grass species *Ammophila breviligulata* (Beach Grass); and the C_4 grass *Andropogon gerardii* (Big Bluestem). Appendix 1 lists the oxygen, carbon, and nitrogen isotope compositions of pollen, and the locations for all species analyzed in this study, between years 2009 to 2012. Appendix 2 lists the same information for trees (*Quercus* and *Acer*) analyzed from other locations in North America. The oxygen and carbon isotope results for cellulose from trees, grasses, and marsh species sampled in 2011 are listed in Appendix 3.

4.1 Oxygen isotope geochemistry of pollen at the Pinery, Zurich, and London, Ontario

The average oxygen isotope compositions of pollen from the combined sample of all species in 2009 is significantly different than other years, at $P < 0.05$ (Table 4.1a); however, the average $\delta^{18}O$ values of 2010, 2011, and 2012 do not differ significantly from each other.

4.1.1 $\delta^{18}O$ values of pollen from 2009 to 2012 for tree species from the Pinery, Ontario

The oxygen isotope compositions for pollen from individual trees of *P. resinosa*, *P. strobus*, and *Q. rubra* over the period of 2009 to 2012 are illustrated in Figure 4.1. The four-year average oxygen isotope compositions for each species show little variation; *Q.*

Table 4.1 Statistical analysis using one way ANOVA and Tukey test of differences in pollen isotopic compositions for all tree, grass, and marsh species considered collectively, among years: (a) $\delta^{18}O$ ($F(3,78)=4.801$, $P=0.004$), (b) $\delta^{13}C$ ($F(3,78)=0.046$, $P=0.987$), and (c) $\delta^{15}N$ ($F(3,78)=1.535$, $P=0.212$).

(a)

$\delta^{18}O$	2010	2011	2012
2009	0.019*	0.006*	0.003*
2010		0.988	0.969
2011			0.999

(b)

$\delta^{13}C$	2010	2011	2012
2009	0.997	1.000	1.000
2010		0.994	0.983
2011			1.000

(c)

$\delta^{15}N$	2010	2011	2012
2009	0.908	0.961	0.324
2010		0.994	0.610
2011			0.364

* Significantly different at $P < 0.05$.

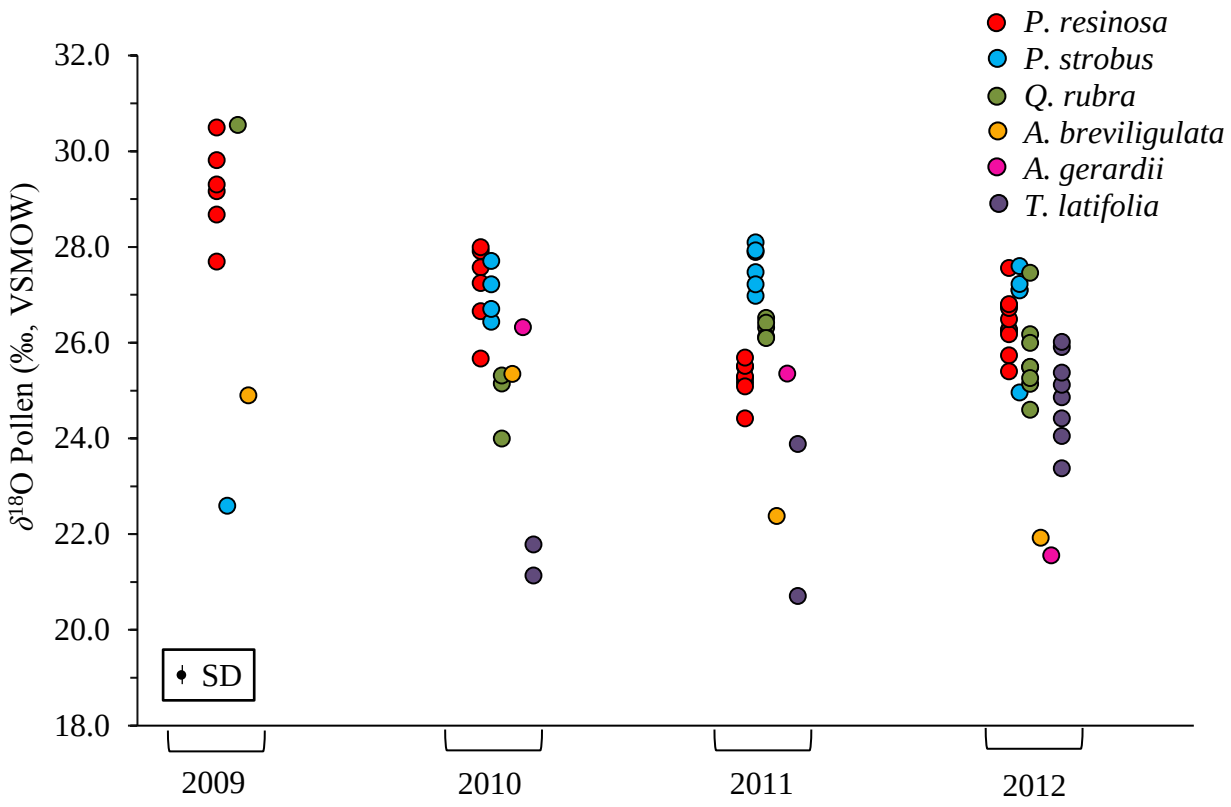

Figure 4.1 Oxygen isotope composition from 2009 to 2012 of pollen for *P. resinosa*, *P. strobus*, *Q. rubra*, *A. breviligulata*, *A. gerardii*, and *T. latifolia*.

rubra (n=17) has the lowest average δ^{18}O value (+26.0 ± 1.4‰) whereas *P. strobus* has the highest average δ^{18}O value (+26.9 ± 1.4‰, n=16).

Species that are significantly different from each other in oxygen isotopic composition are indicated in Table 4.2; there were insufficient δ^{18}O results for pollen from tree species in 2009 for statistical evaluation. No one tree species has consistently higher or lower δ^{18}O values from year to year; in 2009, *P. resinosa* and *Q. rubra* have much higher δ^{18}O values than *P. strobus*, whereas in 2011, *P. strobus* δ^{18}O values are significantly higher than *P. resinosa* and *Q. rubra* δ^{18}O values. In 2012, there are no significant differences of δ^{18}O values among species.

4.1.2 δ^{18}O values of pollen from 2009 to 2012 for grass species from the Pinery, Ontario

The δ^{18}O values of pollen from grass species *A. breviligulata* and *A. gerardii* for the period of 2009 to 2012 are illustrated in Figure 4.1. A δ^{18}O value was obtained for each grass species, each year, by the collection of pollen from grass anthers of multiple grass plants in the same area, with the exception of *A. gerardii* in 2009, which yielded no pollen. As a result of the limited sample size, statistical tests could not be used to detect significant differences in oxygen isotope composition between species. Instead, grasses are reported as different in Table 4.2 if their isotopic compositions fall outside the range of species to which they are compared.

The δ^{18}O values of *A. breviligulata* range from +21.9 to +25.4‰ from 2009 to 2012. The δ^{18}O values of *A. gerardii* vary from +21.6 to +26.3‰ over the same period. The δ^{18}O values for both grass species are similar to each other in 2010 and 2012, but differ by 3‰ in 2011.

Table 4.2 Statistical analysis using one way ANOVA and Tukey test of differences in average pollen oxygen isotopic compositions among species at the Pinery in: (a) 2010 ($F(3,11)=34.511$, $P=0.000$), (b) 2011 ($F(3,17)=37.223$, $P=0.000$), and (c) 2012 ($F(3,30)=5.627$, $P=0.003$).

(a) 2010	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.987	0.004*	Not different	Not different	0.000*
P. strobus		0.012*	Different	Not different	0.000*
Q. rubra			Not different	Different	0.002*
A. breviligulata				Different	Different
A. gerardii					Different

* Significantly different at $P < 0.05$.

** Sample size for this species was too small for statistical analysis; instead, a response of "Different" indicates that the measurement for that year does not fall within the range of $\delta^{18}O$ values of the species to which it is being compared, whereas a response of "Not different" indicates that it does.

Table 4.2 Continued.

(b) 2011	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.000*	0.000*	Not different	Different	0.608
P. strobus		0.002*	Different	Not different	0.372
Q. rubra			Different	Not different	0.479
A. breviligulata				Different	Different
A. gerardii					Different

* As in part (a) of this table. ** As in part (a) of this table.

Table 4.2 Continued.

(c) 2012	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.855	0.313	Different	Different	0.014*
P. strobus		0.128	Different	Different	0.009*
Q. rubra			Different	Different	0.589
A. breviligulata				Different	Different
A. gerardii					Different

* As in part (a) of this table. ** As in part (a) of this table.

4.1.3 $\delta^{18}O$ values of pollen from 2009 to 2012 for cattails from Zurich and London, Ontario

Oxygen isotope compositions for pollen from the cattail species *T. latifolia* from 2010 to 2012 are illustrated in Figure 4.1. Average $\delta^{18}O$ values range from +21.5‰ in 2010 to +24.9‰ in 2012. The $\delta^{18}O$ values of pollen from *T. latifolia* show little variation in 2010 (± 0.5‰) but vary considerably in 2011 (± 2.2‰). Significant differences between *T. latifolia* and other species are summarized in Table 4.2, with statistical significance at $P < 0.05$.

4.2 Oxygen isotope geochemistry of leaf and stem cellulose from the Pinery, Ontario

Tissue samples from stems and leaves were collected in 2011 from a single plant of each species of *P. resinosa, P. strobus, Q. rubra, A. breviligulata, A. gerardii*, and *T. latifolia*; pollen from the same individuals was also collected and analyzed, as described in Chapter 3. The $\delta^{18}O$ values of cellulose from stems and leaves collected during pollen fly are illustrated together with those of pollen in Figure 4.2.

The average $\delta^{18}O$ value of 2011 pollen for *P. resinosa* (+25.2‰) is significantly lower ($P < 0.05$) than the average cellulose $\delta^{18}O$ value of its needles (+28.6‰) and stems (+28.9‰). The average $\delta^{18}O$ value of pollen for *P. strobus* (+27.6‰) is also significantly lower than the average cellulose $\delta^{18}O$ values of its needles (+28.7‰) and stems (+28.1‰), at $P < 0.05$. The average $\delta^{18}O$ value of pollen for *Q. rubra* (+26.3‰) is not significantly different from that of leaf cellulose (+25.5‰); however, this pollen average $\delta^{18}O$ value is significantly lower than the average stem cellulose $\delta^{18}O$ value (+28.2‰). The pollen $\delta^{18}O$ value for *A. breviligulata* (+22.4‰) is much lower than the

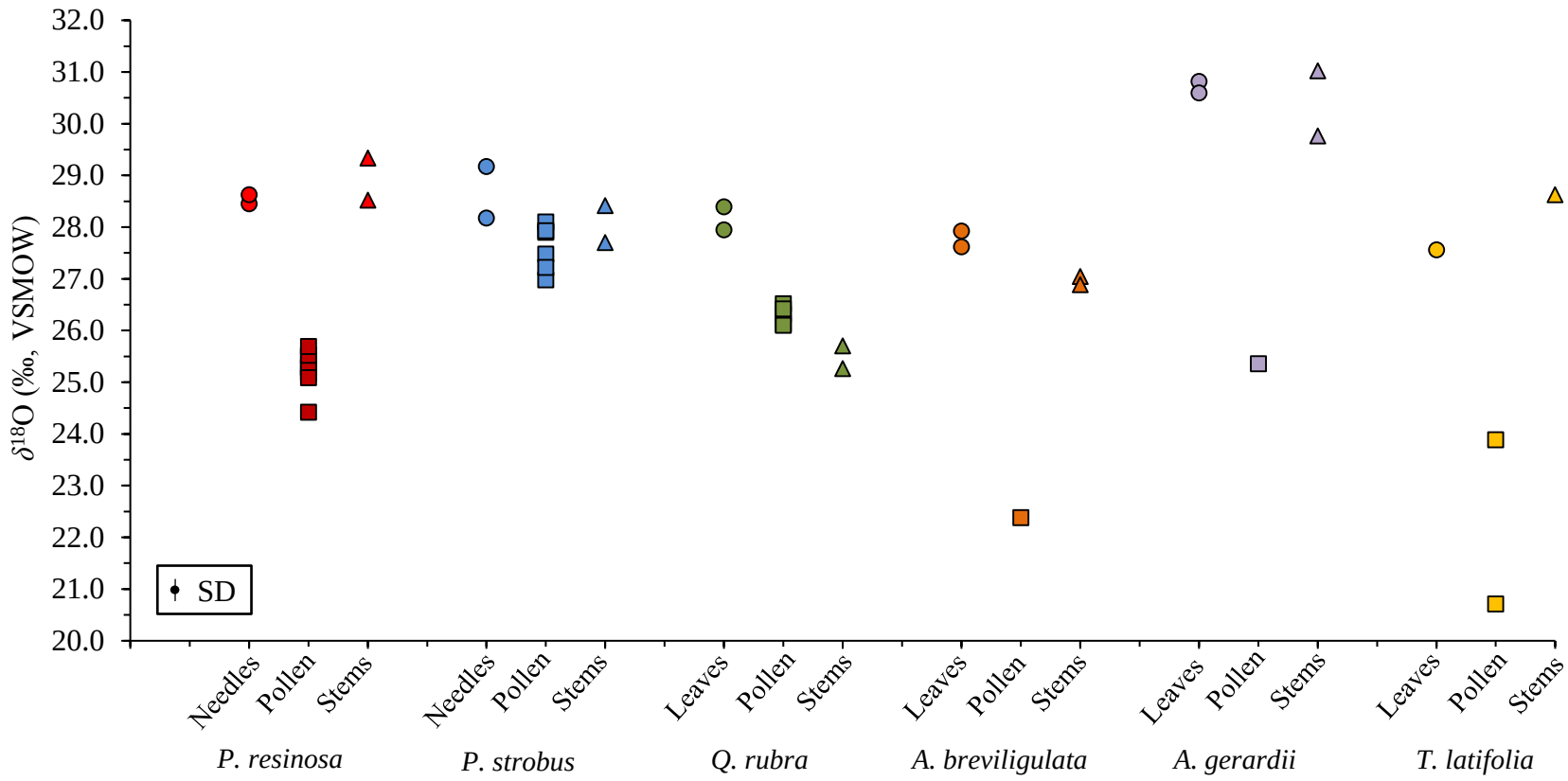

Figure 4.2 Comparison of oxygen isotope compositions of pollen (squares) and cellulose from needles/leaves (circles) and stems (triangles) collected at pollen fly in 2011 at the Pinery.

average cellulose $\delta^{18}O$ value of its leaves (+27.8‰) and stems (+27.0 ‰). The average $\delta^{18}O$ value of pollen for *A. gerardii* (+25.4‰) is also much lower than the average cellulose $\delta^{18}O$ value of its leaves (+30.7‰) and stems (+29.9‰). The average $\delta^{18}O$ value of pollen for *T. latifolia* (+22.3‰) is significantly lower than the average cellulose $\delta^{18}O$ value of its leaves (+27.6‰) and stems (+28.6‰).

Tissue samples of stems and leaves for which the average $\delta^{18}O$ values are listed above were obtained from the same plant early (May) and late (October) in the growing season for all species except *T. latifolia*. Smaller, and likely younger leaves were selected in October, to represent late-season growth. The oxygen isotopic compositions of cellulose from these samples are listed in Table 4.3, and illustrated in Figure 4.3. Several species show little variation in cellulose $\delta^{18}O$ values between samples collected early and late in the growing season: *P. resinosa, A. breviligulata,* and *A. gerardii* differed by 1.0‰ or less. Cellulose $\delta^{18}O$ values of *Pinus strobus* needles increase by 1.6‰ from early to late in the growing season. *Quercus rubra* leaf cellulose shows the largest increase (4.3‰) between early and late in the growing season (Fig. 4.3).

4.3 Oxygen isotope geochemistry of plant water from the Pinery, Ontario

The oxygen isotope compositions of water extracted from different plant parts collected in 2011 and 2012 are summarized in Table 4.4 for *P. resinosa, P. strobus, Q. rubra, A. breviligulata,* and *A. gerardii*.

Table 4.3 Oxygen isotope compositions of stem and leaf cellulose for tree and grass species at the Pinery for 2011, comparing early (May) and late (October) season samples.

Species	Plant Part	$\delta^{18}O$ ‰ (VSMOW) Early Cellulose[1]	$\delta^{18}O$ ‰ (VSMOW) Late Cellulose	$\delta^{13}C$ ‰ (VPDB) Early Cellulose	$\delta^{13}C$ ‰ (VPDB) Late Cellulose
P. resinosa	Needle	+28.6	+28.7	–27.7	–27.2
P. resinosa	Stem	+28.9	+28.7	–27.2	–26.2
P. strobus	Needle	+28.7	+29.9	–27.5	–26.9
P. strobus	Stem	+28.1	+28.5	–27.5	–27.4
Q. rubra	Leaf	+28.2	+32.3	–28.4	–25.6
Q. rubra	Stem	+25.5	+25.6	–27.9	–25.6
A. breviligulata	Leaf	+27.8	+26.4	–25.6	–24.8
A. breviligulata	Stem	+27.0	+26.2	–26.0	–25.6
A. gerardii	Leaf	+30.7	+29.6	–11.7	–11.9
A. gerardii	Stem	+30.4	+28.8	–12.6	–12.0

[1] 'Early' $\delta^{18}O$ values are an average (n=2) of measurements reported in Table 3.5. Locations are as reported in Table 3.5.

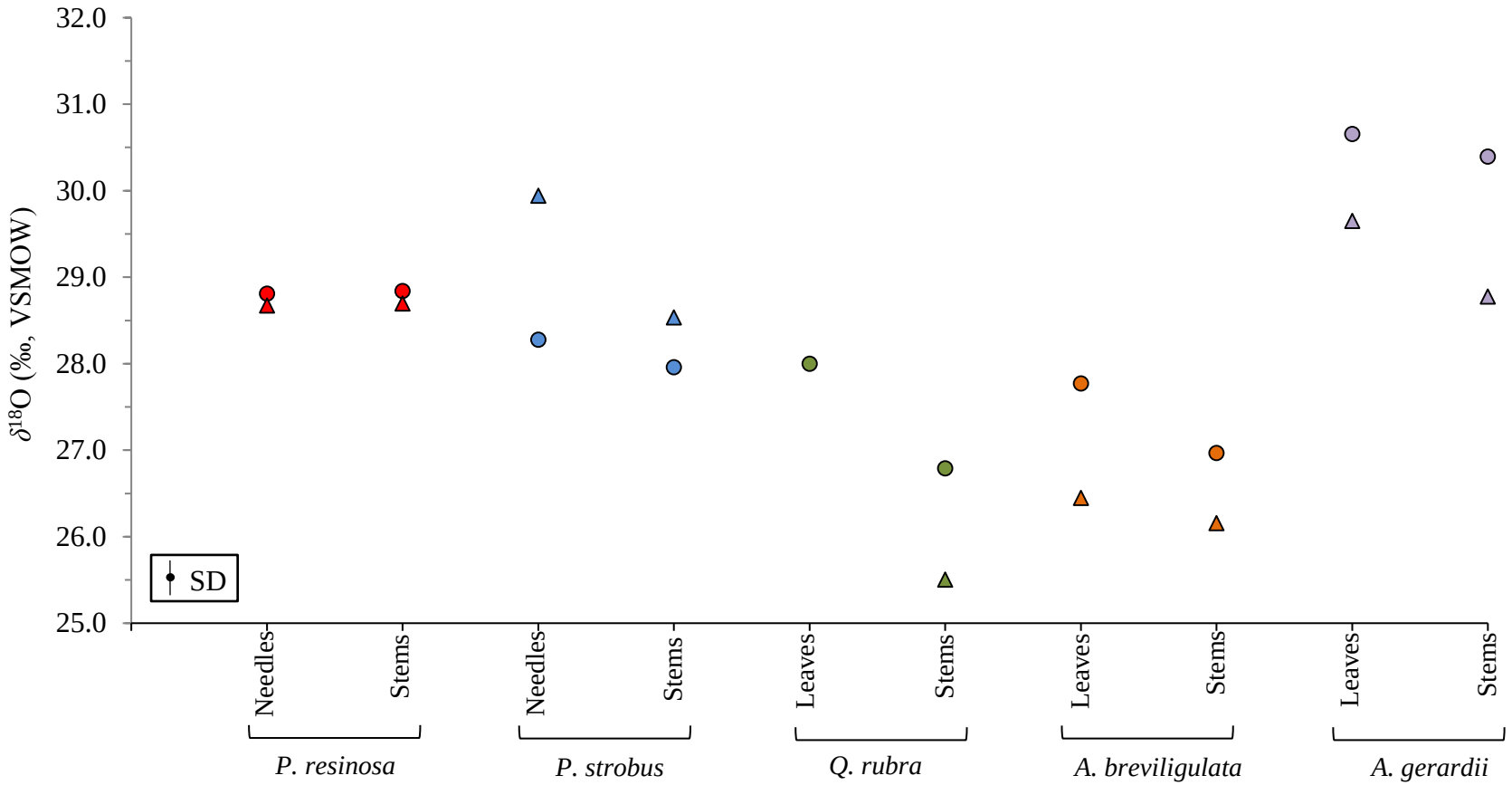

Figure 4.3 Oxygen isotope compositions of cellulose for early (May; circles) and late (October; triangles) season samples during 2011.

4.4 Carbon isotope composition of pollen from the Pinery, Zurich, and London, Ontario

Considered collectively, the carbon isotope compositions of pollen from all species show no significant differences amongst years at $P < 0.05$ (Table 4.1b). The average annual $\delta^{13}C$ values for all C_3 species vary only slightly from as high as –26.9‰ in 2009 to as low as –27.5‰ in 2010 and 2011.

4.4.1 $\delta^{13}C$ values of pollen from 2009 to 2012 for tree species from the Pinery, Ontario

The carbon isotope compositions of *P. resinosa*, *P. strobus*, and *Q. rubra* pollen from 2009 to 2012 are illustrated in Figure 4.4. A comparison of the average $\delta^{13}C$ values (2009 to 2012, inclusive) of each species show little variation; *P. resinosa* (n=30) has the lowest average $\delta^{13}C$ value (–27.6 ± 0.7‰). *Pinus strobus* (n=16) has an average $\delta^{13}C$ value of –27.4 ± 1.4‰, and *Q. rubra* (n=17) has an average $\delta^{13}C$ value of –26.3 ± 0.8‰.

Table 4.5 reports the results of statistical tests for significant differences in pollen carbon isotope composition among species. The sample size was too small to test for significant differences in the carbon isotope data for pollen from tree species in 2009. The 2009 pollen $\delta^{13}C$ values are: *P. strobus* –29.5‰ (n=1); *Q. rubra* –27.3‰ (n=1), and *P. resinosa* –26.6‰ (n=6).

4.4.2 $\delta^{13}C$ values of pollen from 2009 to 2012 for grass species from the Pinery, Ontario

The carbon isotopic composition of pollen collected from Pinery grasses from 2009 to 2012 was determined, except for *A. gerardii*, which did not yield pollen in 2009. The $\delta^{13}C$ values range from –26.5 to –25.3‰ for *A. breviligulata*, and –12.9 to –11.3‰ for *A. gerardii* (Fig. 4.4). For both species, pollen $\delta^{13}C$ values are highest in 2010 and lowest in 2011.

4.4.3 $\delta^{13}C$ values of pollen from 2009 to 2012 for cattails from Zurich and London, Ontario

The $\delta^{13}C$ values of *T. latifolia* pollen from 2010 to 2012 show little variation among years, ranging from –29.4‰ in 2010 to –28.5‰ in 2012 (Fig. 4.4). The least amount of variation in $\delta^{13}C$ values occurs for samples collected in 2011, and is highest in 2010 and 2012. The average $\delta^{13}C$ value of *T. latifolia* pollen is significantly lower than most other C_3 species analyzed in this study (Table 4.5). To determine if the carbon and oxygen isotope compositions of pollen are positively correlated as is reported for tree ring cellulose (Roden et al., 2005), the $\delta^{13}C$ and $\delta^{18}O$ values are compared in Figure 4.5, and show a weak positive correlation ($r = 0.29$, $p < 0.05$).

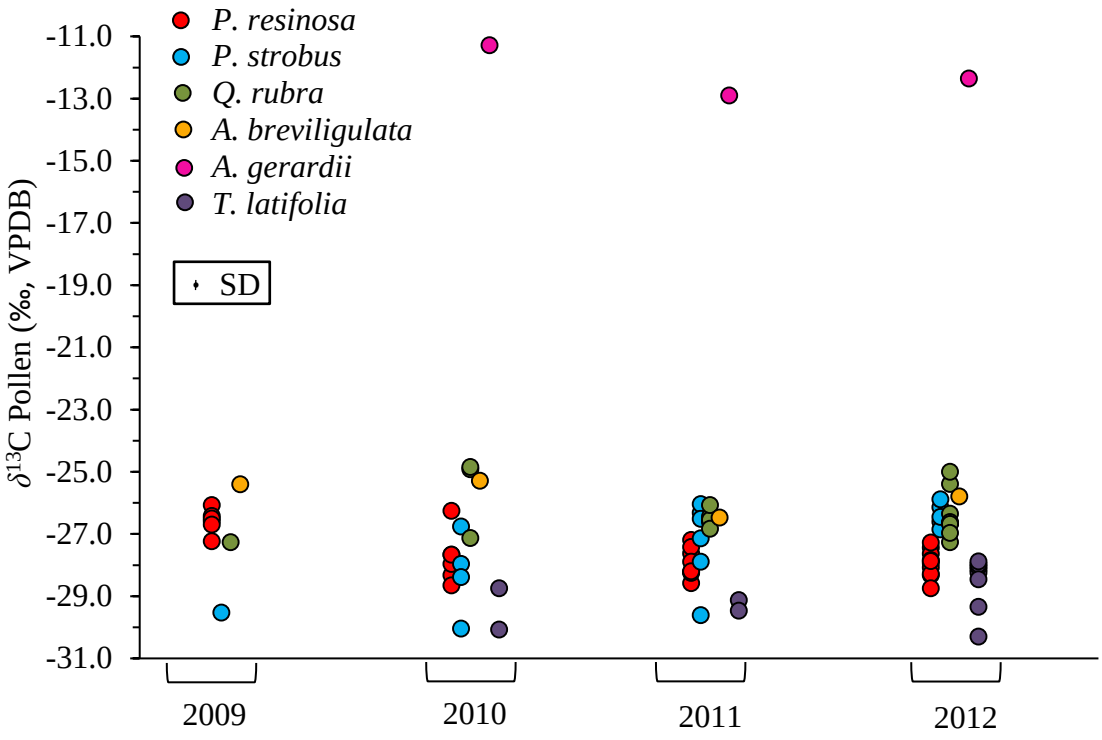

Figure 4.4 Carbon isotope composition from 2009 to 2012 of pollen for *P. resinosa*, *P. strobus*, *Q. rubra*, *A. breviligulata*, *A. gerardii*, and *T. latifolia*.

Table 4.4 Oxygen isotope compositions of plant water for tree and grass species collected during 2011 and 2012, at 5:00 AM and 1:00PM.

Sample Name	Location	Date	Plant Part	$\delta^{18}O$ ‰ (VSMOW) Plant Water
P. resinosa				
11RP-01Br-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 11, 2011	Branch	–7.9
11RP-02Ne-AM	43˚17’02.9076”N 81˚48’15.4836”W	May 27, 2011	Needle	–2.8
11RP-03Ne-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 27, 2011	Needle	–8.1
11RP-04Ne-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 27, 2011	Needle	–2.8
11RP-05St-AM	43˚17’02.9076”N 81˚48’15.4836”W	May 27, 2011	Stem	–7.9
11RP-06Br-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 27, 2011	Branch	–6.6
11RP-07Ne-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 31, 2011	Needle	–9.5
11RP-08St-AM	43˚17’02.9076”N 81˚48’15.4836”W	May 31, 2011	Stem	–6.3
11RP-09St-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 31, 2011	Stem	–6.0
11RP-10Ne-PM	43˚17’02.9076”N 81˚48’15.4836”W	Oct. 15, 2011	Needle	–4.4
11RP-11Br-PM	43˚17’02.9076”N 81˚48’15.4836”W	Oct. 15, 2011	Branch	-–2.2
12RP-01Ne-AM	43˚17’02.9076”N 81˚48’15.4836”W	May 24, 2012	Needle	–0.6
12RP-02Ne-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 24, 2012	Needle	–13.9
12RP-03Br-AM	43˚17’02.9076”N 81˚48’15.4836”W	May 24, 2012	Branch	–7.8
12RP-04Br-PM	43˚17’02.9076”N 81˚48’15.4836”W	May 24, 2012	Branch	–7.9
P. strobus				
11WP-01Br-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 11, 2011	Branch	–11.4
11WP-02Ne-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Needle	–6.6
11WP-03Ne-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Needle	–3.6

Table 4.4 Continued.

Sample Name	Location	Date	Plant Part	$\delta^{18}O$ ‰ (VSMOW) Plant Water
P. strobus				
11WP-04St-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Stem	–6.8
11WP-05Ne-AM	43˚17’07.8000”N 81˚48’08.8020”W	May 31, 2011	Needle	–2.1
11WP-06Ne-PM	43˚17’07.8000”N 81˚48’08.8020”W	May 31, 2011	Needle	–8.9
11WP-07St-AM	43˚17’07.8000”N 81˚48’08.8020”W	May 31, 2011	Stem	–6.4
11WP-08St-PM	43˚17’07.8000”N 81˚48’08.8020”W	May 31, 2011	Stem	–6.2
11WP-09Ne-PM	43˚17’07.8000”N 81˚48’08.8020”W	Oct. 15, 2011	Needle	–2.7
11WP-10St-PM	43˚17’07.8000”N 81˚48’08.8020”W	Oct. 15, 2011	Stem	–9.8
12WP-01Ne-AM	43˚17’07.8000”N 81˚48’08.8020”W	May 24, 2012	Needle	–1.5
12WP-02Ne-PM	43˚17’07.8000”N 81˚48’08.8020”W	May 24, 2012	Needle	–14.8
12WP-03Br-PM	43˚17’07.8000”N 81˚48’08.8020”W	May 24, 2012	Branch	–9.9
12WP-04Br-PM	43˚17’07.8000”N 81˚48’08.8020”W	May 24, 2012	Branch	–8.0
Q. rubra				
11Oak-01Lvs-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Leaf	–7.5
11Oak-02Lvs-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Leaf	–3.6
11Oak-03St-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Stem	–7.2
11Oak-04St-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 27, 2011	Stem	–6.8
11Oak-06Lvs-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 31, 2011	Leaf	–7.6
11Oak-07Lvs-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Leaf	–1.4
11Oak-08Lvs-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Leaf	–1.4
11Oak-09St-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Stem	–11.7

Table 4.4 Continued.

Sample Name	Location	Date	Plant Part	$\delta^{18}O$ ‰ (VSMOW) Plant Water
Q. rubra				
11Oak-10St-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Stem	–10.0
11Oak-11Ba-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Bark	–10.2
11Oak-12Ba-PM	43˚15’52.2684”N 81˚48’57.0636”W	Oct. 15, 2011	Bark	–10.6
12Oak-01Lvs-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Leaf	–5.6
12Oak-02Lvs-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Leaf	–5.2
12Oak-03St-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Stem	–10.3
12Oak-04St-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Stem	–10.8
12Oak-05Br-AM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Branch	–10.3
12Oak-06Br-PM	43˚15’52.2684”N 81˚48’57.0636”W	May 24, 2012	Branch	–11.0
A. breviligulata				
11AM-01Lvs-AM	43˚16’08.2416”N 81˚49’57.5004”W	Aug. 3, 2011	Leaf	–0.9
11AM-02Lvs-PM	43˚16’08.2416”N 81˚49’57.5004”W	Aug. 3, 2011	Leaf	–1.5
11AM-03St-AM	43˚16’08.2416”N 81˚49’57.5004”W	Aug. 3, 2011	Stem	–7.1
11AM-04St-PM	43˚16’08.2416”N 81˚49’57.5004”W	Oct. 15, 2011	Stem	–5.3
11AM-05Lvs-PM	43˚16’08.2416”N 81˚49’57.5004”W	Oct. 15, 2011	Leaf	–1.1
A. gerardii				
11BB-01Lvs-AM	43˚16’06.0348”N 81˚49’47.3412”W	Aug. 3, 2011	Leaf	–0.7
11BB-02Lvs-PM	43˚16’06.0348”N 81˚49’47.3412”W	Aug. 3, 2011	Leaf	+0.5
11BB-03St-AM	43˚16’06.0348”N 81˚49’47.3412”W	Aug. 3, 2011	Stem	–4.3
11BB-04St-PM	43˚16’06.0348”N 81˚49’47.3412”W	Aug. 3, 2011	Stem	–5.5

Table 4.4 Continued.

Sample Name	Location	Date	Plant Part	δ^{18}O ‰ (VSMOW) Plant Water
Q. rubra				
11BB-05Lvs-PM	43˚16’06.0348”N 81˚49’47.3412”W	Oct. 15, 2011	Leaf	+0.2
11BB-06St-PM	43˚16’06.0348”N 81˚49’47.3412”W	Oct. 15, 2011	Stem	–9.1

4.5 Carbon isotope composition of stem and leaf cellulose from the Pinery, Ontario

The carbon isotope compositions of cellulose sampled in 2011 from stems and leaves during pollen fly are illustrated in Figure 4.6. Within a species, the pollen δ^{13}C values differ significantly ($P < 0.05$) from cellulose δ^{13}C values only for *Q. rubra* and *T. latifolia* (Appendix 3).

The carbon isotope compositions of cellulose show little variation between tissues sampled early (May) versus late (October) in the growing season (Fig. 4.7, Table 4.3). The δ^{13}C values of all species and plant parts increase in over the growing season by less than 1.0‰ except for *P. resinosa* needles and *Q. rubra* stems, which decrease in δ^{13}C during the late growing season by 0.9‰ and 0.7‰, respectively (Fig. 4.7).

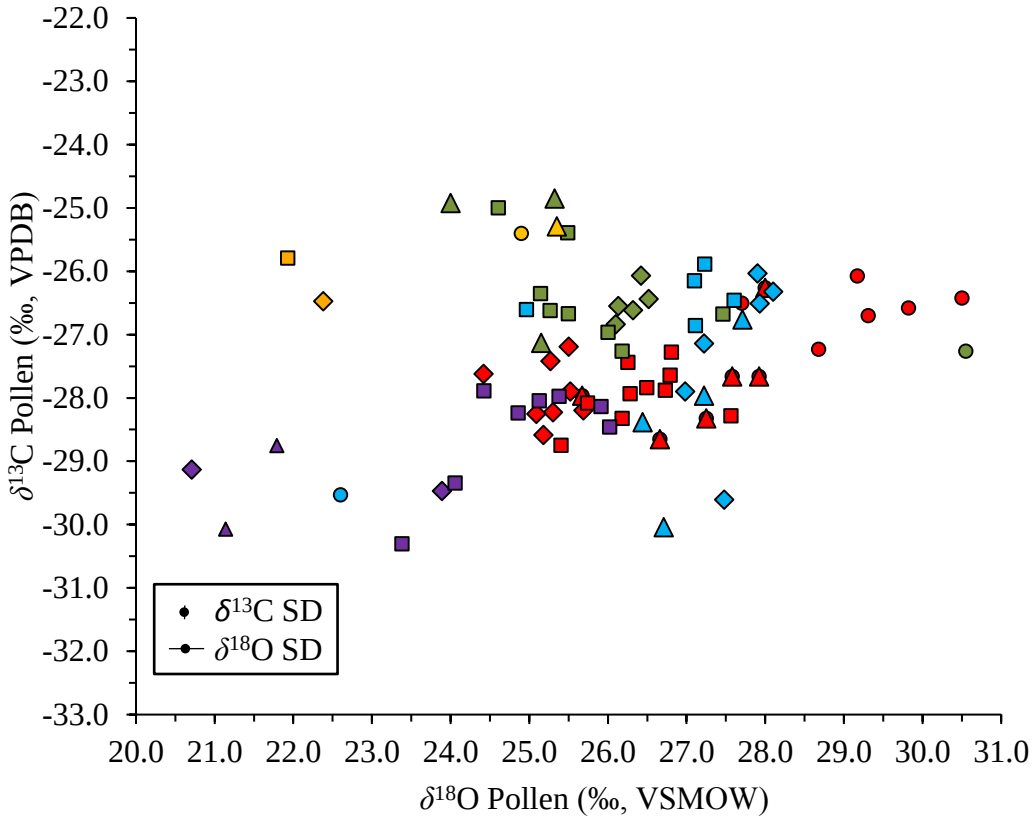

Figure 4.5 Carbon versus oxygen isotope compositions from 2009 (circles), 2010 (triangles), 2011 (diamonds), and 2012 (squares) of pollen for *P. resinosa* (red), *P. strobus* (blue), *Q. rubra* (green), *A. breviligulata* (yellow), and *T. latifolia* (purple), at the Pinery, Zurich, and London, Ontario.

4.6 C/N ratios of pollen

Carbon and nitrogen percentages and ratios for all pollen are reported in Appendix 1. In this study, coniferous species (*P. resinosa* and *P. strobus*) have average C:N ratios of 26 and 25 respectively, and the deciduous *Q. rubra* have an average C:N ratio of 9, consistent with those reported in the literature. *Ammophila breviligulata* and *A. gerardii* have C:N ratios of 14 and 15 respectively, and *T. latifolia* a ratio of 18. The average C:N ratio of *Quercus* and *Acer* species sampled in the United States of America is 11 and 9 respectively.

4.7 Nitrogen isotope composition of pollen from 2009 to 2012, from the Pinery, Zurich, and London, Ontario

Collectively, the nitrogen isotope compositions of pollen from all species show no significant differences among years (Table 4.1c). The average pollen δ^{15}N value for all species is lowest in 2009 (–3.6‰); however, *T. latifolia* was not sampled during this year, and this species contributes to the higher average δ^{15}N values in other years. The average pollen δ^{15}N value of all species, including *T. latifolia*, ranges from –2.9‰ in 2011 to –0.5‰ in 2012.

4.7.1 δ^{15}N values of pollen from 2009 to 2012 for tree species from the Pinery, Ontario

The nitrogen isotope compositions of tree pollen between 2009 and 2012 are illustrated in Figure 4.8. Some species differ significantly in δ^{15}N value from each other, but there is no consistent pattern from year to year (Table 4.6). The nitrogen isotopic variation

within species is apparently larger in 2011 and 2012 than 2009 and 2010, but sample size also increases from 2009 to 2012.

4.7.2 δ^{15}N values of pollen from 2009 to 2012 for grass species from the Pinery, Ontario

The nitrogen isotope compositions for pollen from the grass species *A. breviligulata* and *A. gerardii* between 2009 and 2012 are illustrated in Figure 4.8. The δ^{15}N values of *A. breviligulata* pollen are >0‰ and increase each year from 2009 to 2012. The δ^{15}N values of *A. gerardii* pollen are <0‰, and decrease by 1.8‰ from 2011 to 2012.

4.7.3 δ^{15}N values of pollen from 2009 to 2012 for cattails from Zurich and London, Ontario

Nitrogen isotope compositions for pollen of *T. latifolia* collected from Zurich and London, Ontario, from 2010 to 2012 are illustrated in Figure 4.8. Average pollen δ^{15}N values of *T. latifolia* are consistently much higher than all other species; for example, 15.2‰ higher than the average δ^{15}N value of *P. resinosa* in 2010 (Figure 4.8). From 2010 to 2012, average *T. latifolia* pollen δ^{15}N values decrease by an average of 1.6‰ per year.

Table 4.5 Statistical analysis using one way ANOVA and Tukey test of differences in average pollen carbon isotopic compositions among all species at the Pinery in: (a) 2010 (F(3,11)=5.581, P=0.014), (b) 2011 (F(3,17)=6.942, P=0.003), and (c) 2012 (F(3,27)=21.078, P=0.000).

(a) 2010	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.873	0.078	Different	Different	0.300
P. strobus		0.038*	Different	Different	0.647
Q. rubra			Not different	Different	0.014*
A. breviligulata				Different	Different
A. gerardii					Different

* Significantly different at $P < 0.05$.

** Sample size for this species was too small for statistical analysis; instead, a response of "Different" indicates that the measurement for that year does not fall within the range of $\delta^{18}O$ values of the species to which it is being compared, whereas a response of "Not different" indicates that it does.

Table 4.5 Continued

(b) 2011	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.426	0.028*	Different	Different	0.168
P. strobus		0.430	Not different	Different	0.027*
Q. rubra			Not different	Different	0.003*
A. breviligulata				Different	Different
A. gerardii					Different

* As in Table 4.8a. ** As in Table 4.8a.

Table 4.5 Continued

(c) 2012	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.001*	0.000*	Different	Different	0.232
P. strobus		1.000	Not different	Different	0.000*
Q. rubra			Not different	Different	0.000*
A. breviligulata				Different	Different
A. gerardii					Different

* As in Table 4.8a. ** As in Table 4.8a.

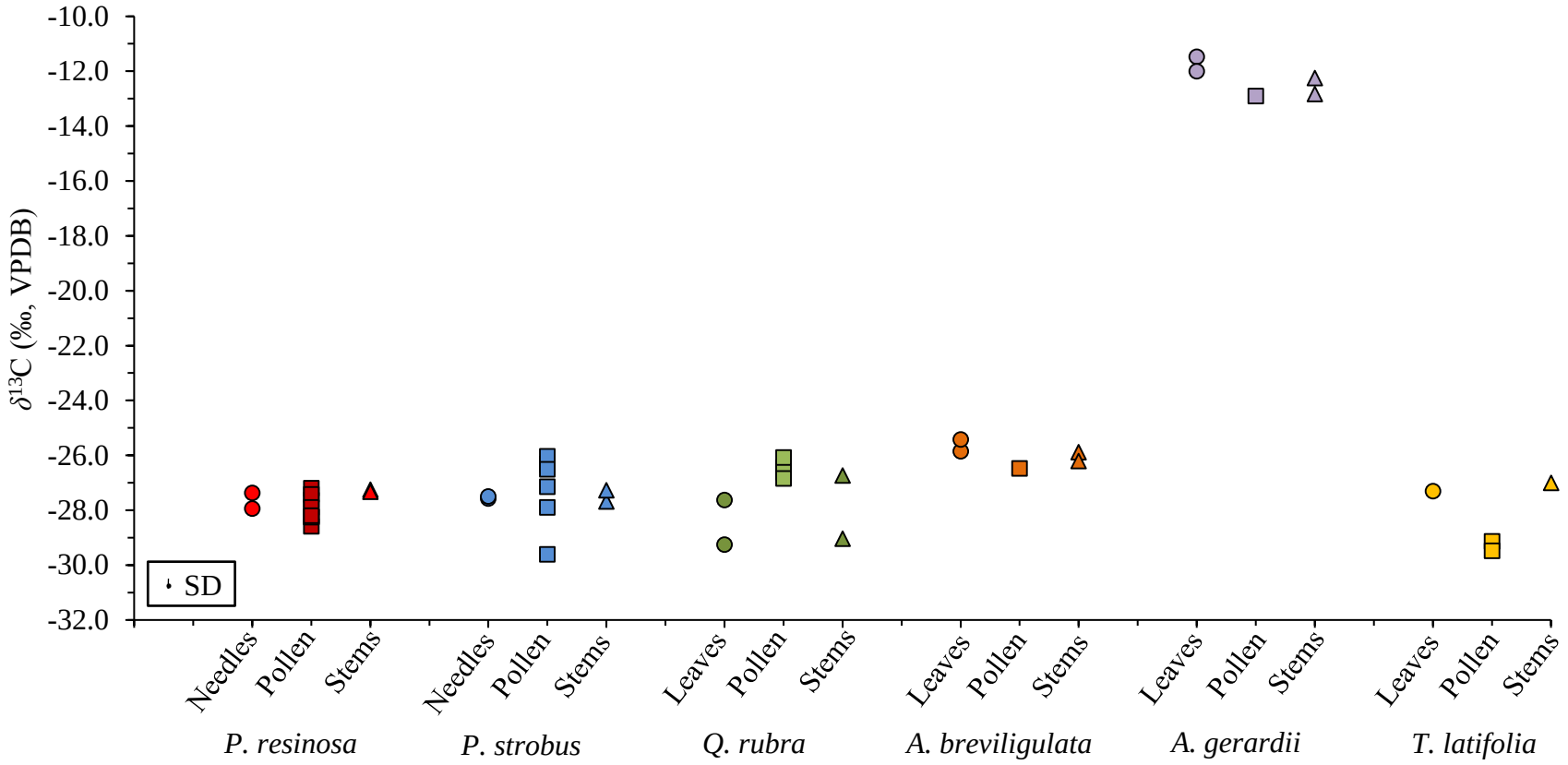

Figure 4.6 Carbon isotope compositions of pollen (squares) and cellulose from needles/leaves (circles) and stems (triangles) for 2011.

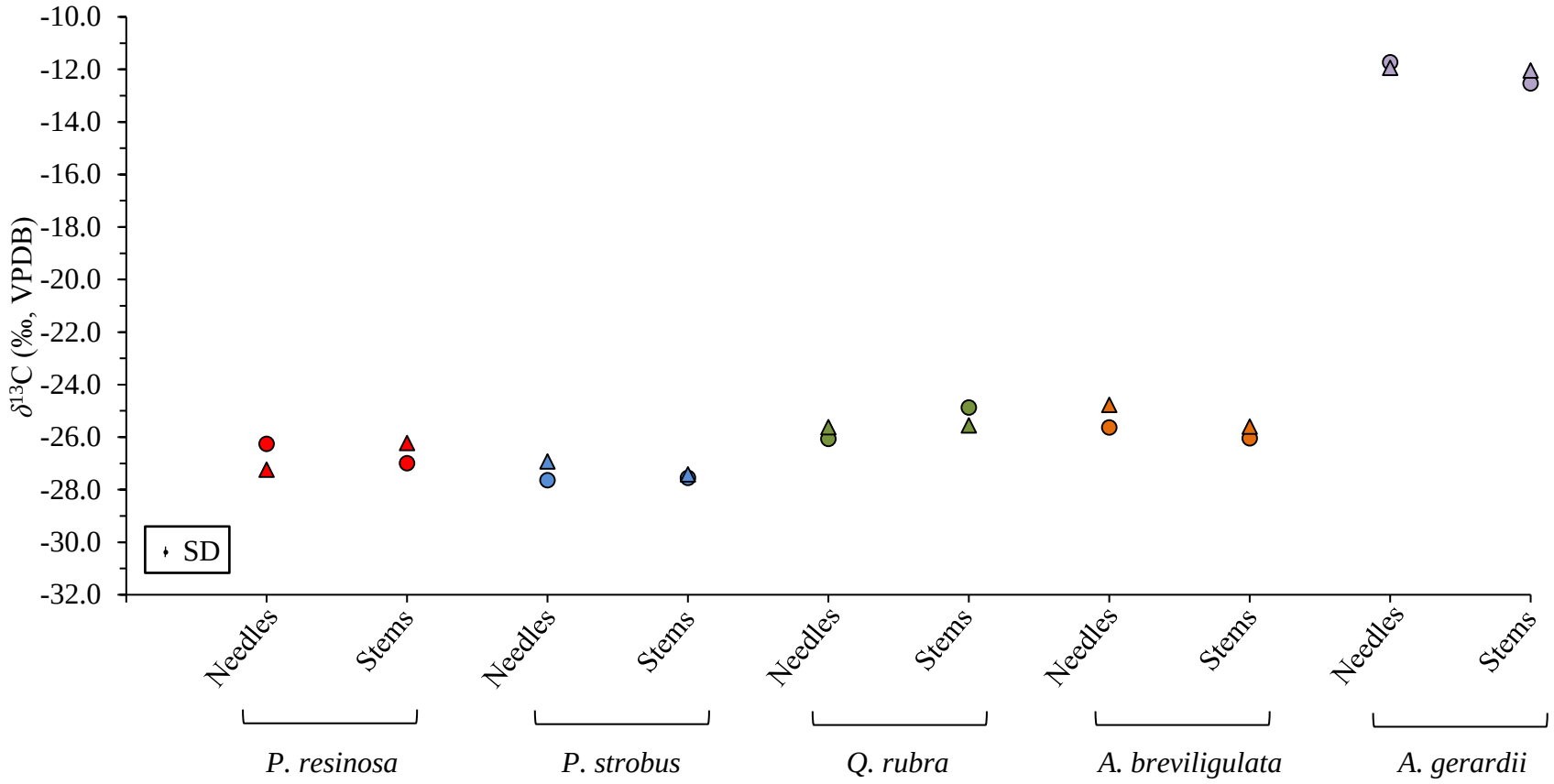

Figure 4.7 Carbon isotope compositions of cellulose from early (May; circles) season sampling compared with late (October; triangles) season sampling for 2011.

Table 4.6 Statistical analysis using one way ANOVA and Tukey test of differences in average pollen nitrogen isotopic compositions among all species at the Pinery in: (a) 2010 (F(3,11)=239.874, P=0.000), (b) 2011 (F(3,17)=83.417, P=0.000), and (c) 2012 (F(3,28)=172.018, P=0.000).

(a) 2010	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.814	0.104	Different	Different	0.000*
P. strobus		0.405	Different	Different	0.000*
Q. rubra			Different	Different	0.000*
A. breviligulata				Different	Different
A. gerardii					Different

* Significantly different at $P < 0.05$.

** Sample size for this species was too small for statistical analysis; instead, a response of "Different" indicates that the measurement for that year does not fall within the range of $\delta^{18}O$ values of the species to which it is being compared, whereas a response of "Not different" indicates that it does.

Table 4.6 Continued.

(b) 2011	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.965	0.891	Different	Not different	0.000*
P. strobus		0.702	Different	Different	0.000*
Q. rubra			Different	Not different	0.000*
A. breviligulata				Different	Different
A. gerardii					Different

* As in Table 4.9a. ** As in Table 4.9a.

Table 4.6 Continued.

(c) 2012	*P. strobus*	*Q. rubra*	***A. breviligulata*	***A. gerardii*	*T. latifolia*
P. resinosa	0.211	0.315	Different	Not different	0.000*
P. strobus		0.018*	Different	Different	0.000*
Q. rubra			Different	Not different	0.000*
A. breviligulata				Different	Different
A. gerardii					Different

* As in Table 4.9a. ** As in Table 4.9a.

4.8 Isotopic composition of tree pollen from selected sites in North America for 2011 and 2012

4.8.1 $\delta^{18}O$ values of tree pollen

The oxygen isotope compositions of tree pollen for 2011 and 2012 from the North American sites are illustrated in Figure 4.9. When the *Quercus* genus was not available for sampling, *Acer* was substituted; both species have similar horizontal-spreading root systems in mature trees, and likely access water from the same soil depth (Lyford, 1980; Lyford and Wilson, 1964). The expected $\delta^{18}O$ values for precipitation during October and November of the previous growing season for each location are also illustrated in Figure 4.9 (and listed in Appendix 2), as calculated following Bowen and Revenaugh (2003). With the exception of the Texas location in 2011, pollen $\delta^{18}O$ values generally vary in a fashion consistent with precipitation $\delta^{18}O$ values.

4.8.2 $\delta^{13}C$ values of tree pollen

The carbon isotope compositions of tree pollen for 2011 and 2012 from the North American sites are illustrated in Figure 4.10. These data are compared to average relative humidity (as a measure of aridity) expected during pollen formation for that location. While such correlations have been noted in previous studies, there is no such relationship apparent in the present study.

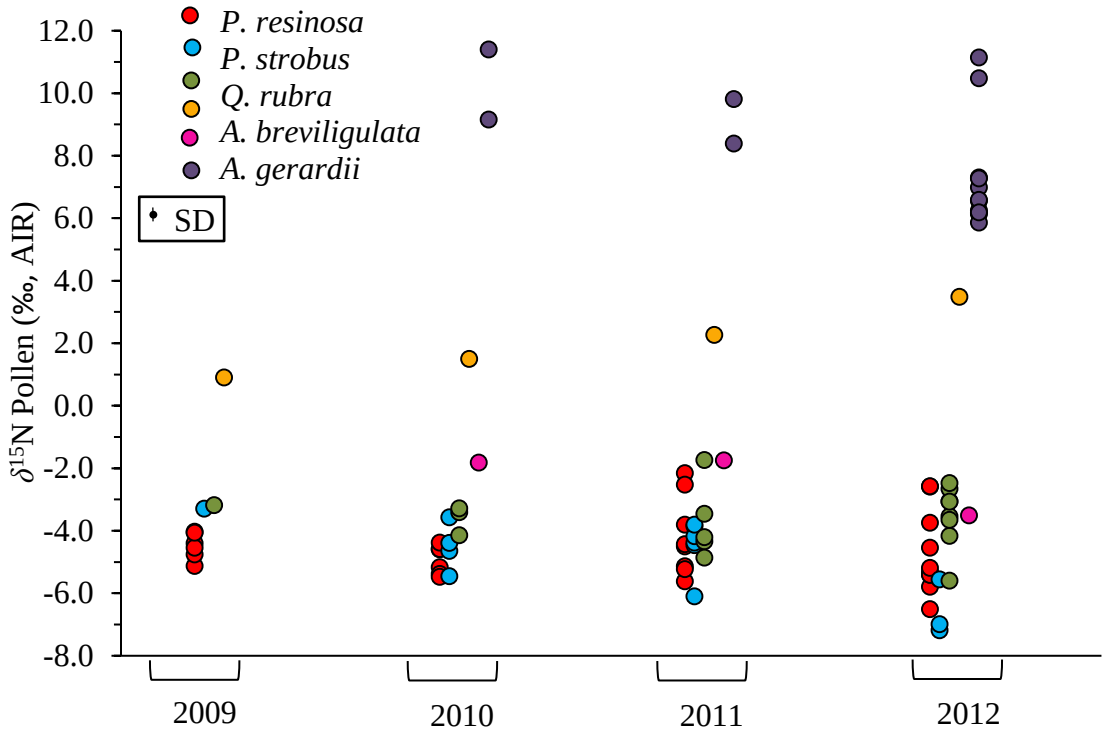

Figure 4.8 Nitrogen isotope composition from 2009 to 2012 of pollen for *P. resinosa*, *P. strobus*, *Q. rubra*, *A. breviligulata*, *A. gerardii*, and *T. latifolia*.

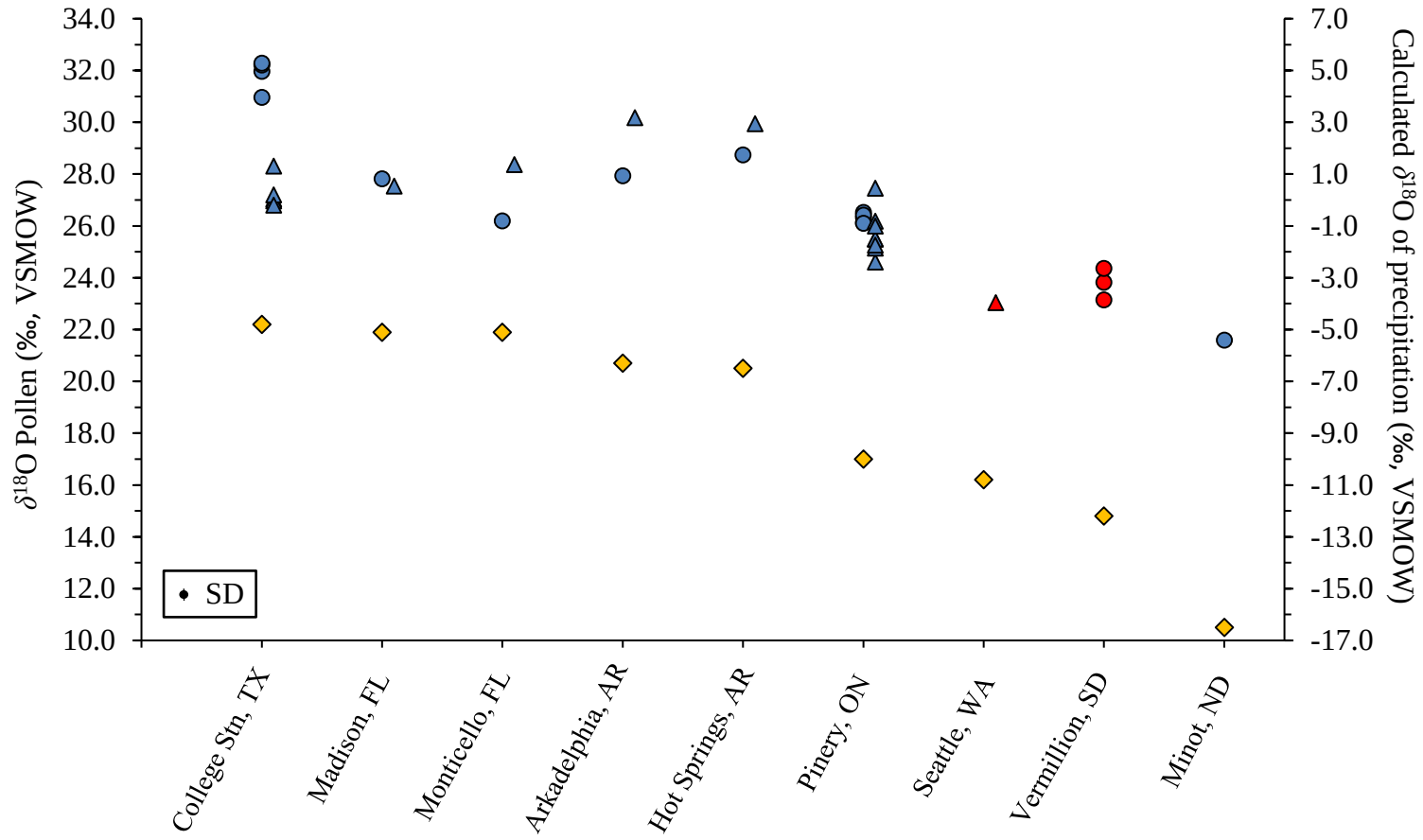

Figure 4.9 Oxygen isotope compositions of pollen for *Quercus* (blue) and *Acer* (red) genera from North American sites for 2011 (circles) and 2012 (triangles), compared to the oxygen isotope composition of precipitation (yellow diamonds). Sites are arranged from highest to lowest calculated precipitation $\delta^{18}O$ values for each location, as calculated following Bowen and Revenaugh (2003).

4.8.3 δ^{15}N values of tree pollen

The nitrogen isotope compositions of tree pollen for 2011 and 2012 from the North American sites are illustrated in Figure 4.11. The pollen δ^{15}N values from the Pinery are significantly lower than at the other North American sites. At sites where more than one tree was analyzed in the same year, pollen δ^{15}N values vary by as much as 4.8‰ (Texas location, 2011). In 2011, tree pollen from the Texas, South Dakota, and North Dakota sites all had higher δ^{15}N values than other locations.

4.9 Temperature, relative humidity and precipitation data for the Pinery and Goderich, Ontario

Temperature, relative humidity and precipitation data for the Pinery and nearby Goderich, Ontario, are listed in Appendix 4 for the months of March to August (when vegetation is actively growing), and October and November (end of growing season), for 2008 to 2012. These time intervals span the period of pollen formation, including the previous growing season, which influences the isotopic composition of sugars used in tree pollen formation. Data reported are: (i) daily maximum and minimum temperatures, (ii) relative humidity at 1:00pm (optimum growing time for vegetative tissue), and (iii) the amount of daily precipitation. For 2008 to 2011, these data were obtained from Environment Canada's Goderich site, which is located near the Pinery, along the shore of Lake Huron (Figure 1.1), and from the Pinery when available. Data for 2012 was collected directly from the Pinery site. Table 4.7 summarizes environmental data for periods pertaining to the time of sugar formation for trees (October/November), cattails (May/June), and grasses (July).

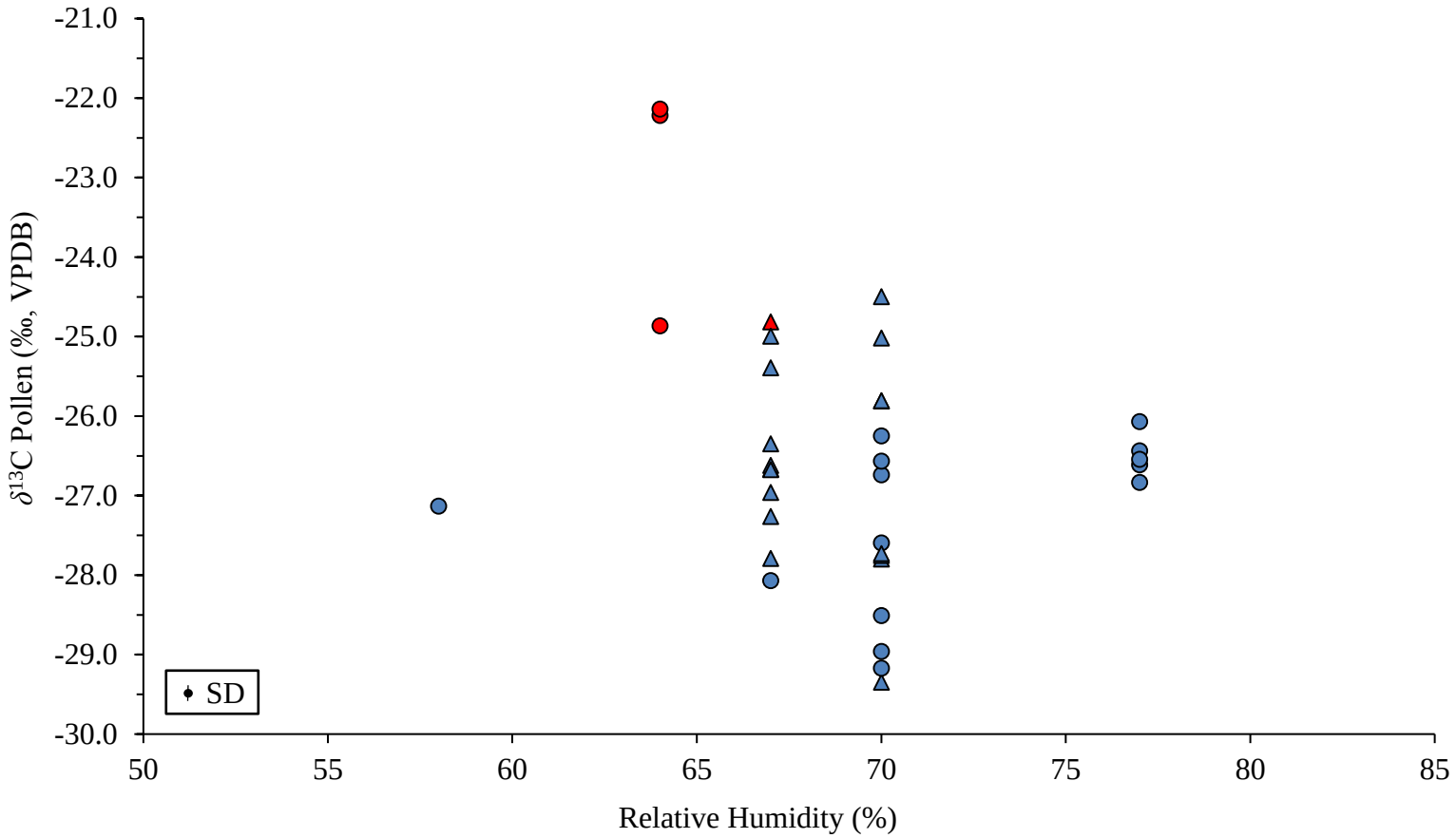

Figure 4.10 Carbon isotope composition of pollen for *Quercus* (blue) and *Acer* (red) genera from North American sites for 2011 (circles) and 2012 (triangles) versus average relative humidity at the time of pollen formation.

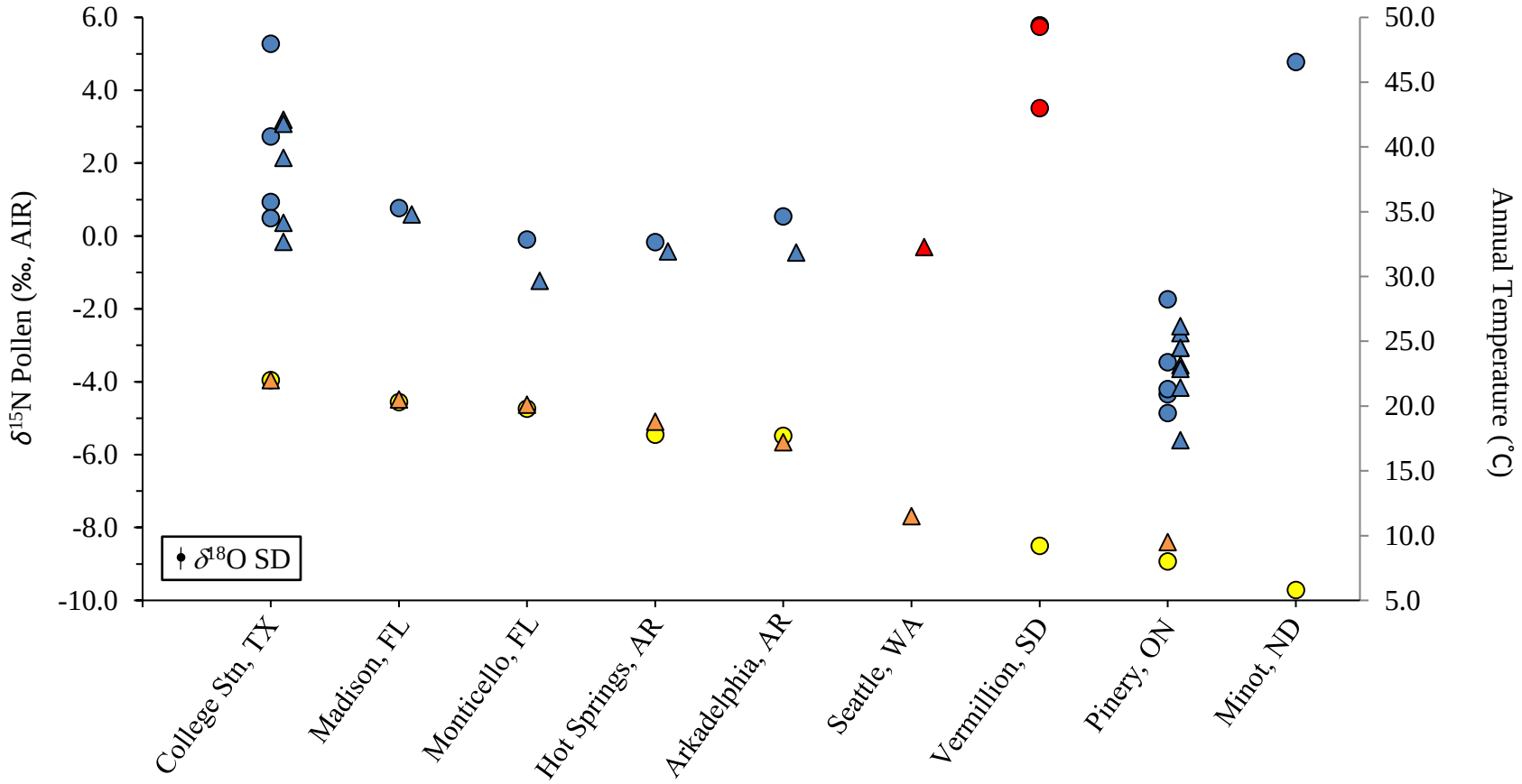

Figure 4.11 Nitrogen isotope compositions of pollen for *Quercus* (blue) and *Acer* (red) genera from North American sites for 2011 (circles) and 2012 (triangles), compared to average annual temperature for 2011 (yellow circles) and 2012 (orange triangles).

Table 4.7 Summary of average temperature, relative humidity, and total precipitation amount for periods when sugars are formed for trees (October/November), cattails (May/June), and grasses (July).

		2008	2009	2010	2011	2012[1]
May/June (affects cattails)	Temperature (˚C)	n/a	13.3	15.4	14.8	18.2
	Relative Humidity (%)	n/a	73	77	76	63
	Precipitation Amount (mm)	n/a	149.6	253.9	222.5	85.4
July (affects grasses)	Temperature (˚C)	n/a	17.4	20.9	21.2	24.0
	Relative Humidity (%)	n/a	78	79	76	64
	Precipitation Amount (mm)	n/a	48	89.9	34.6	37.8
October/November (affect trees)	Temperature (˚C)	+7.9	8.3	8.2	9.0	n/a
	Relative Humidity (%)	70	68	66	68	n/a
	Precipitation Amount (mm)	289.5	106.7	124.5	184.0	n/a
March-November (entire growing season)	Temperature (˚C)	n/a	12.3	13.7	13.2	17.1
	Relative Humidity (%)	n/a	72	72	73	65
	Precipitation Amount (mm)	n/a	533.5	518.6	715.8	197.5

[1] Growing season for 2012 is calculated from March to August, marking the end of sampling for plants in this study.

Chapter 5

5 Discussion

5.1 Oxygen isotopes

5.1.1 Oxygen isotope composition of pollen at the Pinery, 2009-2012

The oxygen isotope compositions of pollen for tree, marsh, and grass species analyzed in this study, considered collectively, show no statistically significant differences among years (Table 4.4a). Only pollen from 2009 has $\delta^{18}O$ values slightly higher than in 2010, 2011, and 2012. Almost all pollen samples from 2009 with higher $\delta^{18}O$ values than other years are from *P. resinosa*, which hints at a species-specific effect on oxygen isotope composition.

Whether the oxygen isotope composition of pollen is determined by source water or transpired water, or a combination of both, has not been investigated until now. The $\delta^{18}O$ values of plant tissue in general are reported to be mainly reflective of source water, which itself is largely controlled by ambient temperature during precipitation droplet formation and the isotopic composition of the moisture mass in the cloud (Roden and Ehleringer, 2000). The $\delta^{18}O$ values of plant tissue, including pollen, differ from that of the plant's water source because of: (i) fractionation between plant tissue macromolecular components and plant water, and (ii) isotopic fractionation of the plant water relative to source water during transpiration.

Two major components of pollen are sporopollenin and cellulose, which comprise 26% and 10% respectively of the overall dry weight of the entire pollen grain, and almost the entirety of the cell wall. Pollen grains consist of 10-40% (dry weight) proteins, 1-13% lipids (Almeida-Muradian et al., 2005), and 13-55% carbohydrates (Bogdanov, 2004). Proteins and lipids contain little oxygen; also, most oxygen present in proteins and lipids

form carbonyl bonds, which do not easily exchange oxygen isotopes with water in the absence of biochemical reactions that convert carbonyl bonds to other forms. Oxygen from these macromolecules therefore will play only a minimal role in determining the oxygen isotopic composition of whole, dry pollen. Carbohydrates in general have a similar structure to cellulose, which itself is a type of polymeric carbohydrate. In this thesis, the cellulose-water oxygen isotopic fractionation factor is assumed to apply across all carbohydrates present in pollen. The cellulose-water fractionation factor is commonly reported to be +27 ±3‰ (Yakir and DeNiro, 1990; Sternberg, 1989); some studies, however, suggest a mean value that is up to 3‰ lower (Sternberg et al., 2006). The degree to which the oxygen isotope composition of cellulose is fractionated from plant water is likely a result of species-specific biosynthetic pathway effects during cellulose synthesis (Sternberg et al., 2006).

In addition to the cellulose-water oxygen isotopic discrimination, the fractionation factor between sporopollenin and water will also contribute to the overall $\delta^{18}O$ value of pollen. The fractionation factor between sporopollenin and water has not yet been determined directly. Sporopollenin is composed of a mixture of biopolymers such as carotenoids and carotenoid esters (Brooks and Shaw, 1978), and its oxygen is likely fractionated during formation. In cellulose production, when glucose is converted to fructose bisphosphate, the hydroxyl attached at its carbon-2 location is converted to a carbonyl, thus exposing it to isotope exchange during the conversion (Sternberg et al., 2006). In sporopollenin formation, a carbonyl bond is formed in the production of a tetraketide intermediate, and this same bond is converted to a hydroxyl bond in the final formation step, thereby allowing for the possibility of isotopic exchange at this site (Grienenberger et al., 2010). Because the amount of oxygen present in exchangeable hydroxyl and carbonyl bonds is similar for sporopollenin and cellulose, we predict that sporopollenin will have a similar fractionation factor to cellulose.

In addition to isotopic fractionation from water during molecular synthesis, the $\delta^{18}O$ values of plant tissue are also strongly influenced by transpiration. Source water, beginning with precipitation, determines the initial isotope composition of oxygen

utilized in tissues; some ^{18}O enrichment of precipitation, however, can occur because of evaporation prior to root uptake. That said, the oxygen isotopic composition of soil water is not fractionated further during root uptake (Ehleringer and Dawson, 1992). Once this water reaches the leaves, however, transpiration causes the plant water to become further enriched in ^{18}O, resulting in tissues with higher $\delta^{18}O$ values (Barbour, 2007). The degree to which the oxygen isotopic composition of pollen is controlled by source water, evaporation, and transpiration is assessed next by comparison of results for cattails, dune grasses, and trees (both coniferous and deciduous), all from the Pinery and nearby areas.

5.1.2 Oxygen isotope composition of cattails from Zurich, Ontario, 2010-2012

Within the context of the current study, one important way in which cattails (*T. latifolia*) differ from trees is that at the end of each growing season, the above-ground portion of the plant (leaves, stems, and flowers) dies, leaving only a subterranean mass called a rhizome (underground stem). The rhizome remains dormant during the winter, and stores nutrients for use in early spring, when stalks and leaves begin to grow anew. Once growth is initiated and leaves form, photosynthetic activity begins, and quickly becomes the source of energy for the plant.

In Zurich and London, Ontario, *T. latifolia* pollen is produced within the plant in late May and early June, and released in late June. Figures 5.1 to 5.4 compare the difference between calculated $\delta^{18}O$ values of cattail water (measured pollen $\delta^{18}O$ values minus 27‰) and the $\delta^{18}O$ values of precipitation ($\Delta^{18}O_{precipitation}$; Table 4.1) with the range of relative humidity measured for the study area in the years of sampling. In 2010 and 2011, values of $\Delta^{18}O_{precipitation}$ were only slightly higher than 0‰. This is expected because *T. latifolia* pollen formation utilizes source water which itself derives almost directly from precipitation. The tendency towards slightly positive $\Delta^{18}O_{precipitation}$ values may be explained by minor evaporation of the ponded water in which *T. latifolia*

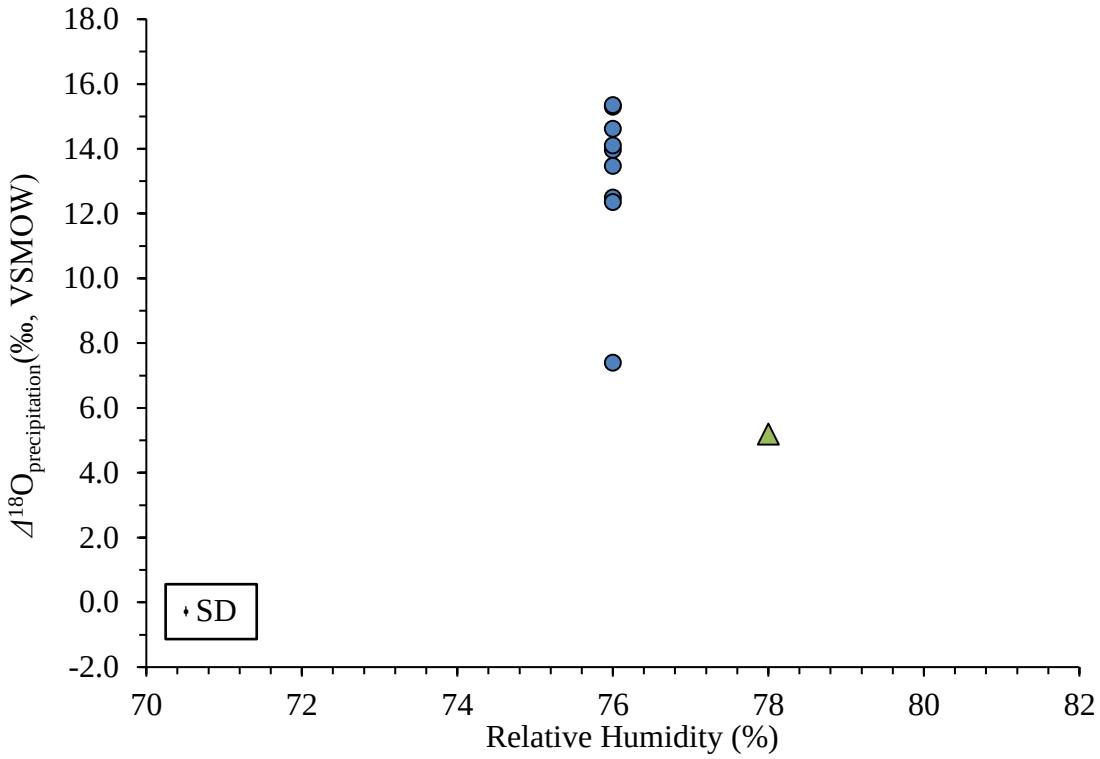

Figure 5.1 $\delta^{18}O_{calc\ plant\ water}$ - $\delta^{18}O_{precipitation}$ ($\Delta^{18}O_{precipation}$) versus relative humidity for grasses (triangle/green) and trees (circle/blue) in 2009. Weather data utilized were: July (grasses) and October and November of the previous growing season (trees). Standard deviation of $\Delta^{18}O$ is ±0.2‰.

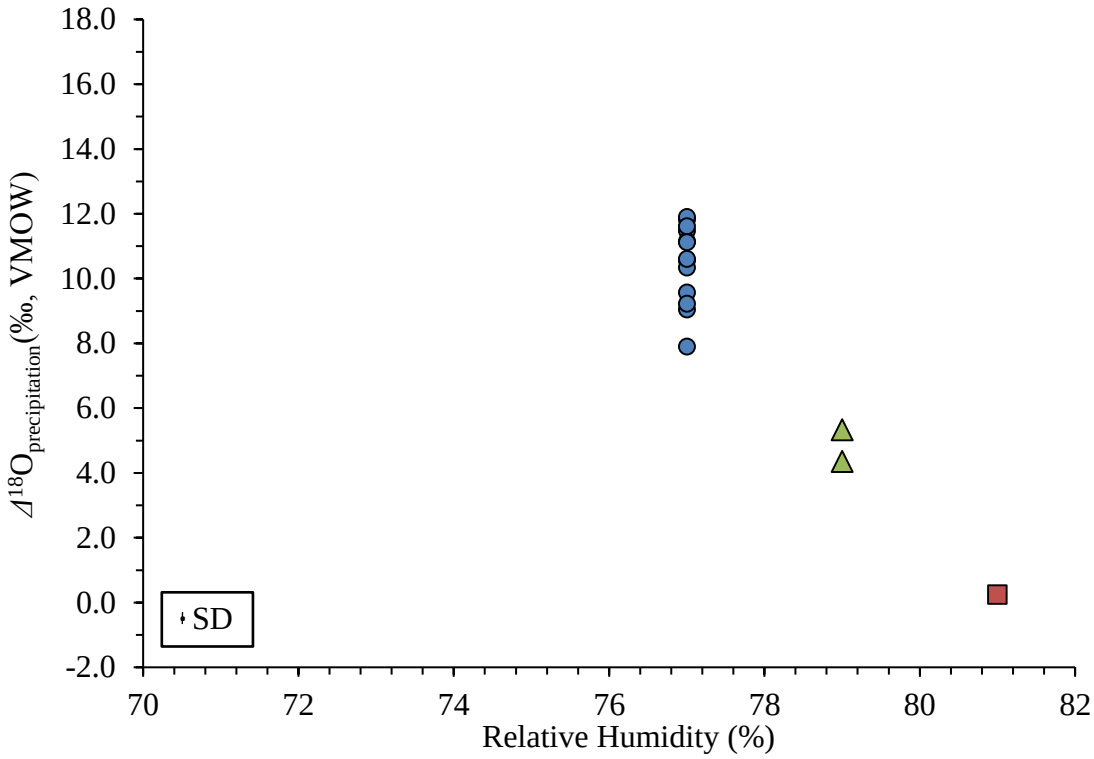

Figure 5.2 $\delta^{18}O_{calc\ plant\ water}$ - $\delta^{18}O_{precipitation}$ ($\Delta^{18}O_{precipation}$) versus relative humidity for cattails (square/red), grasses (triangle/green) and trees (circle/blue) in 2010. Weather data utilized were: October-November of the previous growing season (trees), June (cattails), and July (grasses). Standard deviation of $\Delta^{18}O$ is ±0.2‰.

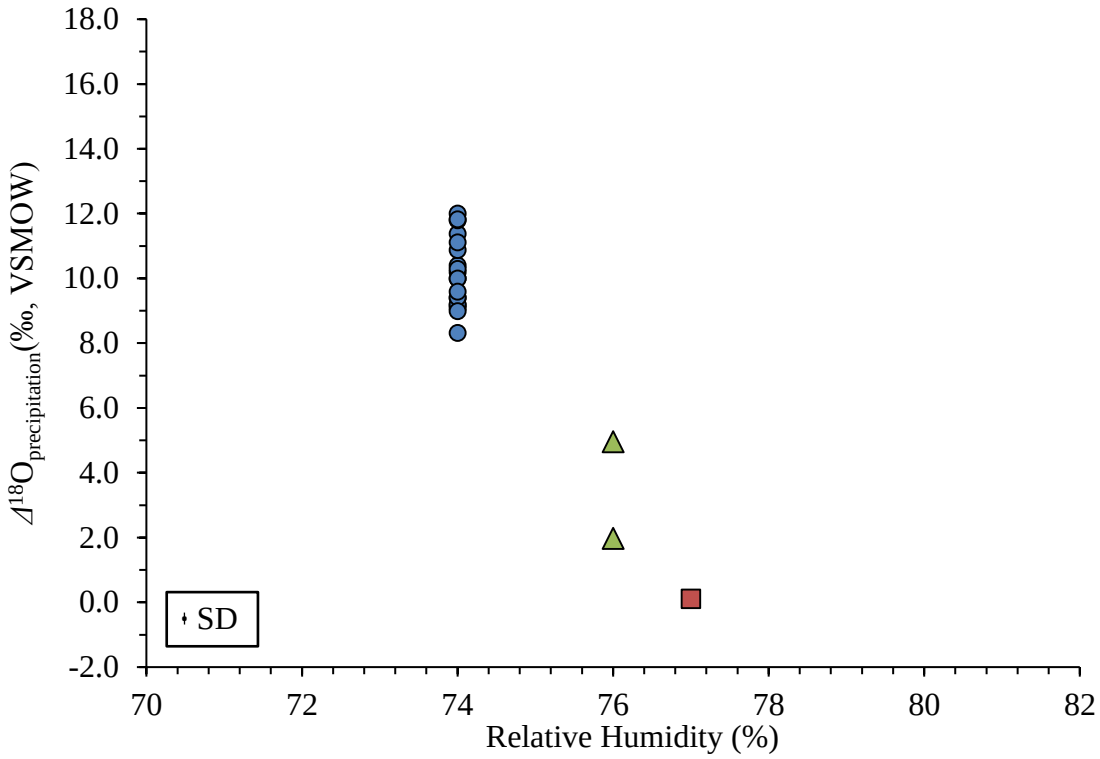

Figure 5.3 $\delta^{18}O_{calc\ plant\ water}$ - $\delta^{18}O_{precipitation}$ ($\Delta^{18}O_{precipation}$) versus relative humidity for cattails (square/red), grasses (triangle/green) and trees (circle/blue) in 2011. Weather data utilized were: October-November of the previous growing season (trees), June (cattails), and July (grasses). Standard deviation of $\Delta^{18}O$ is ±0.2‰.

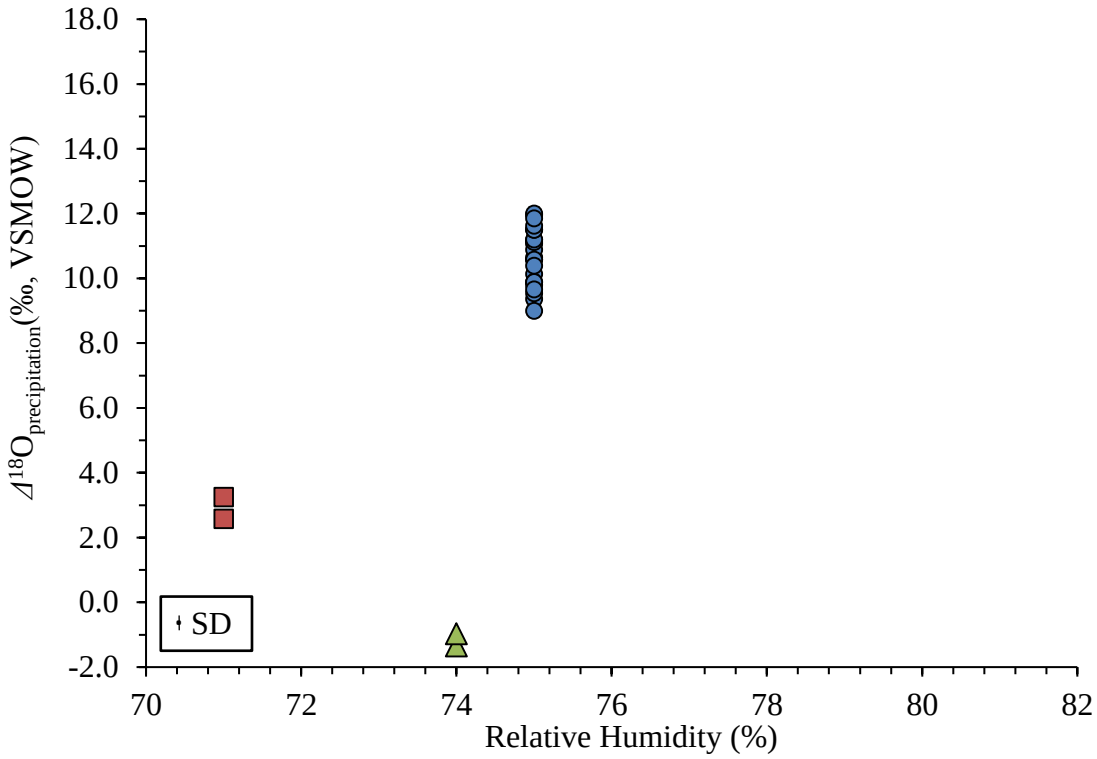

Figure 5.4 $\delta^{18}O_{calc\ plant\ water}$ - $\delta^{18}O_{precipitation}$ ($\Delta^{18}O_{precipation}$) versus relative humidity for cattails (square/red), grasses (triangle/green) and trees (circle/blue) in 2012. Weather data utilized were: October-November of the previous growing season (trees), June (cattails), and July (grasses). Standard deviation of $\Delta^{18}O$ is ±0.2‰.

grows, causing slight ^{18}O enrichment prior to root water uptake. In 2012, relative humidity during pollen formation (63%) was lower than in 2010 (77%) and 2011 (76%). The larger value of $\Delta^{18}O_{precipitation}$ for 2012 (~ +3.0‰) relative to the other years is consistent with lower relative humidity and hence greater evaporation. Because $\Delta^{18}O_{precipation}$ values for *T. latifolia* are close to 0‰, we conclude that the oxygen isotopic composition of *T. latifolia* pollen is not greatly influenced by transpiration or evaporation. The absence of such effects may be explained by the physiology of the plant. Both leaves and stems in *T. latifolia* are attached at the base of the plant, where they are immersed in water. Sugars formed in the leaf must travel down to the base of the plant via the phloem before travelling back up to the site of pollen synthesis. Turgor gradients within the phloem cause the continuous loading and unloading of photosynthates such as sugar molecules (Münch, 1930; van Bel, 2003). Sugars unloaded from the phloem may exchange with the less ^{18}O-enriched water near the base of the stem before being reloaded into the phloem, thereby decreasing $\delta^{18}O$ values of sugar molecules used in pollen synthesis (Barnard et al., 2007).

In short, after accounting for the previously reported 27‰ cellulose-water fractionation factor, the $\delta^{18}O$ values of *T. latifolia* pollen reflect those of precipitation, with a tendency towards minor ^{18}O-enrichment, attributable to evaporative effects on its source water pool. This result also suggests that the overall sporopollenin-water oxygen isotopic fractionation for *T. latifolia* pollen is similar to that of cellulose.

5.1.3 Oxygen isotope composition of grasses at the Pinery, 2009-2012

For C_3 (*A. breviligulata*) and C_4 (*A. gerardii*) grass species at the Pinery, pollen is manufactured and released in late July to early August. Although grasses do store sugars over the winter, these reserves are consumed completely to initiate growth in early spring, until photosynthetic processes take over (White, 1973). By the time pollen is

released in late July to early August, grasses have long been using sugars produced in the same season for tissue production.

Because pollen in grasses at the Pinery form in the few weeks leading up to pollen fly, it is typically July precipitation that contributes to the $\delta^{18}O$ values of sugars used in pollen production. The average values of $\Delta^{18}O_{precipitation}$ for 2009, 2010 and 2011 are +5.2‰, +5.8‰ and +3.5‰ respectively (Figs. 5.1 to 5.3). These values are higher than those for *T. latifolia*, which suggests a greater degree of ^{18}O-enrichment in plant water than predicted by measured precipitation $\delta^{18}O$ values for this time period (Appendix 3). New growth for *A. breviligulata* and *A. gerardii* begins in May and pollen formation begins late June to early July. As such, leaves are present for up to two months prior to pollen synthesis. Sugars used in pollen formation are formed in these leaves; because leaves are predominantly attached to the stem at the base of the plant, however, the sugars are translocated in the phloem downward to the base of the plant before travelling back up towards the inflorescence (flower), as discussed further below. The average $\Delta^{18}O_{precipitation}$ for grasses in 2012 is –1.1‰. Because relative humidity during pollen formation in 2012 was lower than previous years, it is not a factor contributing to this low value. Further investigation with a larger sampling size is necessary to comment further.

Fractionation of oxygen isotopes can occur at various steps during formation of this pollen. Initially, evaporation of soil water can take place prior to water uptake by the plant, thereby increasing the $\delta^{18}O$ values of water that enters the roots and stem. Once in the leaf, plant water is further enriched in ^{18}O as a result of transpiration. Sugars produced in the leaf using this water then move into the phloem, and are then translocated from this area of high water pressure in the leaf, to areas of lower pressure. In grasses such as *A. breviligulata* and *A. gerardii*, this path leads to the base of the plant before traveling up to the inflorescence. The continuous loading and unloading of photosynthates within the phloem during translocation allows for the interaction of sucrose molecules with lower ^{18}O stem water, and as for *T. latifolia*, associated isotopic exchange may lower the overall $\delta^{18}O$ of sugar molecules travelling to sites of pollen synthesis (Barnard et al., 2007).

In 2010 and 2011, *A. gerardii* had larger $\Delta^{18}O_{precipitation}$ than *A. breviligulata* (Figs. 5.2 and 5.3). This difference may be a result of a greater exchange between sugars and lower ^{18}O stem water in *A. breviligulata*. While the leaves of both grass species predominantly attach at the base of the stem, near or at ground level, in *A. gerardii* there is a greater number of leaves attached along the stem than in *A. breviligulata*. As such, sugars travelling from the leaf in the phloem may travel down the entire length of the stem via turgor pressure once they reach the stem, or travel upwards in the stem to sites of pollen synthesis. Sugars that do not travel downward in the stem do not mix and exchange with lower ^{18}O stem water near the base of the plant, and are thus more enriched in ^{18}O. In 2012, the difference in $\Delta^{18}O_{precipitation}$ between *A. gerardii* and *A. breviligulata* is very small. The reason for this is unknown.

5.1.4 Oxygen isotope composition of trees at the Pinery, 2009-2012

The trees examined in this study differ greatly from cattails and grasses in growth and pathway of sugars during translocation, subsequent to formation in the leaf. In the deciduous tree, *Q. rubra*, pollen is produced prior to foliage growth. Photosynthesis has not yet been initiated, and therefore the sugars required for pollen formation are not produced at the same time. Instead, sugars are accessed from energy reserves in the tree (Cherbuy et al., 2000; Nambiar and Fife, 1991). In needle conifers such as *P. resinosa* and *P. strobus*, processes required for metabolic activities, such as photosynthesis and respiration, decrease rapidly after September. Plant growth ceases during the winter, beginning again with the initiation of photosynthesis in the spring, triggered by signals such as sunlight amount and temperature (McGregor and Kramer, 1963). In short, unlike cattails and grasses wherein foliage is available to produce the sugars contributing to pollen formation, tree species examined in this study utilize resources stored late in the previous growing season to support new spring growth (Cherbuy et al., 2000).

In tree species, sugars are translocated from leaves to the site of pollen synthesis via long, continuous phloem tissues. In the leaf, sugars are actively pumped into the phloem, which forms an extensive network throughout the tree. From this area of high pressure, the sugars are passively mobilized within the phloem to areas of low pressure, where they are actively unloaded (De Schepper et al., 2013). During translocation, resources within the phloem may flow upwards or downwards. They are not required to move to the base of the tree before traveling to the inflorescence or pine cone, unlike what is physiologically necessary in cattails, *A. breviligulata*, and to a somewhat lesser extent, *A. gerardii*. The average $\Delta^{18}O_{precipitation}$ for tree species from 2009 to 2012 in this study range from +10.2 to +13.2‰ (Figs. 5.1 to 5.4). These values suggest that the sugars contributing to pollen formation retain an ^{18}O-rich signature resulting from transpiration in the leaf to a much greater extent than grass species or cattails.

When transpiration increases, the $\delta^{18}O$ values at the site of transpiration also increase, as a result of the higher likelihood of lighter molecules, such as $H_2{}^{16}O$, diffusing through the stomata at a faster rate than heavier molecules, such as $H_2{}^{18}O$. The amount of transpiration increases when diurnal temperature variation increases (Cernusak et al., 2002). In addition, lower relative humidity produces increased transpiration in the leaf (Tullus et al., 2012). In the present study, transpiration strongly affects the oxygen isotope composition of leaf water collected from trees at the same location and at the same time as pollen, depending on time of day (Fig. 5.5). Leaf water collected in 2011 on a day with relatively low diurnal temperature variation and high relative humidity (T = 8.9-14.7˚C, RH = 92%) shows relatively small changes in $\delta^{18}O$ values. In contrast, leaf water collected in 2012 on a day with larger diurnal temperature variation and much lower relative humidity (T = 14.5-31.5˚C, RH = 46%) shows very large and fluctuating daily $\delta^{18}O$ values.

In all woody trees, the entire tree structure serves as a storage organ for sugars used in initial spring growth (Loescher et al., 1990). As such, sugars have a more direct pathway to the site of pollen synthesis; there is little interaction with lower ^{18}O water as it enters

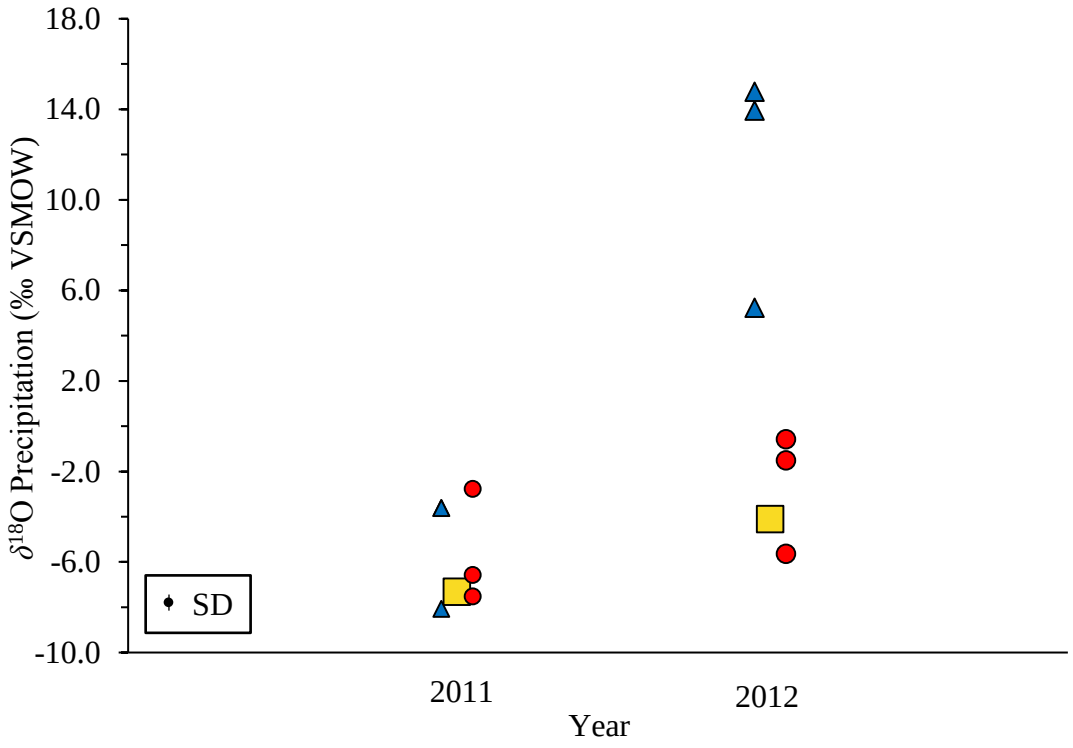

Figure 5.5 Oxygen isotope composition of whole-leaf water collected at the same time and location on the tree as pollen. AM (red circles) = 05:00 hours; PM (blue triangles) = 13:00 hours. The oxygen isotope composition of precipitation collected for May (yellow squares) is also illustrated. Temperature in 2011 is 8.9˚C (AM) and 14.7˚C (PM), and relative humidity is 92%; temperature in 2012 is 14.5˚C (AM) and 31.5˚C (PM), and relative humidity is 46%. Standard deviation of $\delta^{18}O$ is ±0.1‰.

the root. The sugars contributing to pollen formation therefore retain much of the ^{18}O-rich signature acquired because of leaf transpiration.

In general, there are no systematic differences between the pollen δ^{18}O values of the deciduous *Q. rubra* and its coniferous counterparts, *P. resinosa* and *P. strobus*. In 2010 and 2011, the oxygen isotope composition of *Q. rubra* pollen differs from coniferous pollen; however, not in the same direction. This outcome suggests that the oxygen isotope composition of sugars used in pollen synthesis are not controlled by whether the tree is coniferous or deciduous, but by how each species responds to environmental pressures (see section 5.1.4.1).

5.1.4.1 Variation in oxygen isotope composition of pollen within a single species at the Pinery

The standard deviation of δ^{18}O values within individual species varies from year to year (Table 5.1). We focus here on *P. resinosa*, because of its availability for sampling. In 2009, δ^{18}O values of *P. resinosa* pollen show a large variation (+27.7 to +30.5‰; SD ±1.0‰), which contrasts with much less variability and lower δ^{18}O values in 2011 (+24.4 to +25.7‰; SD ±0.4‰). This difference suggests that the oxygen isotope composition of pollen responds to environmental controls that can vary from year to year. The amount of precipitation is likely one such control. Pollen in 2011 was synthesized from sugars produced in 2010, a year having considerably less precipitation than other years. The upper levels of soil in 2010 retained less water, forcing trees to utilize their deeper roots to acquire water. Influenced less by evaporation than near surface water, the deeper groundwater has a relatively consistent and lower average δ^{18}O value within the Pinery (–11.0 ± 0.2‰) relative to soil water (≥ –9.5‰) (Fred Longstaffe, personal communication). *Pinus resinosa* is known to have a root system that penetrates to greater depths than *P. strobus* and *Q. rubra*, and therefore likely utilized less ^{18}O-rich soil water and/or groundwater during this year, thus producing pollen with lower δ^{18}O values.

Table 5.1 Average isotopic compositions of pollen at the Pinery.

Species	Year	Average δ^{18}O ‰ (VSMOW) Pollen	Range (SD) of δ^{18}O ‰ (VSMOW) Pollen	Average δ^{13}C ‰ (VPDB) Pollen	Range (SD) of δ^{13}C ‰ (VPDB) Pollen	Average δ^{15}N ‰ (AIR) Pollen	Range (SD) of δ^{15}N ‰ (AIR) Pollen	N
P. resinosa	2009	+29.2	1.0	–26.6	0.4	–4.5	0.4	6
P. resinosa	2010	+27.2	0.9	–27.8	0.8	–4.9	0.5	6
P. resinosa	2011	+25.2	0.4	–27.9	0.5	–4.2	1.3	8
P. resinosa	2012	+26.4	0.6	–28.0	0.4	–4.7	1.3	10
P. strobus	2010	+27.0	0.6	–28.3	1.4	–4.5	0.8	4
P. strobus	2011	+27.6	0.4	–27.3	1.3	–4.5	0.8	6
P. strobus	2012	+26.8	1.0	–26.4	0.4	–6.6	0.9	5
Q. rubra	2010	+24.8	0.7	–28.3	1.4	–3.6	0.5	3
Q. rubra	2011	+26.3	0.2	–26.5	0.3	–3.7	1.2	5
Q. rubra	2012	+25.7	0.9	–26.4	0.8	–3.5	1.0	8
T. latifolia	2010	+21.5	0.5	–29.4	0.9	+10.3	1.6	2
T. latifolia	2011	+22.3	2.2	–29.3	0.2	+9.1	1.0	2
T. latifolia	2012	+25.2	1.0	–28.6	0.9	+7.3	1.8	11
A. breviligulata	2009	+24.9	n/a	–25.4	n/a	+0.9	n/a	1
A. breviligulata	2010	+25.4	n/a	–25.3	n/a	+1.5	n/a	1
A. breviligulata	2011	+22.4	n/a	–26.5	n/a	+2.3	n/a	1
A. breviligulata	2012	+21.9	n/a	–25.8	n/a	+3.5	n/a	1
A. gerardii	2010	+26.3	n/a	–11.3	n/a	–1.8	n/a	1
A. gerardii	2011	+25.4	n/a	–12.9	n/a	–1.8	n/a	1
A. gerardii	2012	+21.6	n/a	–12.4	n/a	–3.5	n/a	1

The consistent $\delta^{18}O$ value of the groundwater reservoir that *P. resinosa* trees utilized during the relatively dry 2010 season would account for the smaller variation and generally lower $\delta^{18}O$ values of its pollen during 2011, relative to the other years (Table 5.1). In years with more precipitation, upper soil levels retain more water, allowing higher lateral root branchlets to access soil water. This reservoir is more vulnerable to evaporation and hence ^{18}O-enrichment (Gazis and Feng, 2004).

Soil-water evaporation (and also transpiration), and hence its effects on pollen $\delta^{18}O$ values, can be expected to vary in intensity depending on location within the park. The tree with the highest pollen $\delta^{18}O$ values in 2009 (*P. resinosa*) is located on a small hill, beside the Park roadway, with access to full sunlight, whereas the tree having the lowest pollen $\delta^{18}O$ value in 2009 (*P. resinosa*) is on flat ground, approximately 3 m from the roadway, with slightly less access to sunlight. The difference in pollen $\delta^{18}O$ values is most likely the result of greater soil-water evaporation on the hill site. This can occur when down-slope drainage decreases the soil water reservoir, making it more susceptible to evaporation (Ensign et al., 2006). It could also be a result of different proportions of mobile and immobile soil water with distinctively different $\delta^{18}O$ values. Gazis and Feng (2004) report a higher amount of lower ^{18}O in immobile soil water in non-hilly locations compared to hilly locations.

5.1.5 Oxygen isotope composition of pollen from a single location at the Pinery, 2009-2012

Chesson et al. (2013) have proposed that micro-climatic variations in temperature and relative humidity can result in the absence of any apparent patterns in the oxygen isotope composition of pollen from various angiosperms such as the common sunflower (*Helianthus*). In addition, the $\delta^{18}O$ values of outer branchlets of the Australian hoop

pines (*Araucarai cunninghamii*) are significantly different than inner branchlets, likely because of variability in stomatal conductance within the tree (Prasolova et al., 2001).

To test whether variations in $\delta^{18}O$ values of tree pollen at a single location within a microclimatic zone are similar to those across the Pinery, the same trees of *P. resinosa* and *P. strobus* were sampled annually from the same branch over four years. In this way, subtle variations in relative humidity and temperature resulting from site-specific differences in air circulation and sunlight are largely eliminated.

The $\Delta^{18}O_{precipitation}$ value for pollen from a *P. resinosa* tree located at 43˚ 14’ 15.1908” N, 81˚ 52’ 30.3924” W for 2009-2012 is illustrated in Figure 5.6; the analogous data for a *P. strobus* tree (43˚ 15’ 54.8568” N, 81˚ 48’ 50.8068” W) are illustrated in Figure 5.7. Both follow the trend in tree pollen $\delta^{18}O$ values with time observed for other specimens of their species.

The $\delta^{18}O$ values of pollen from *P. resinosa* and *P. strobus* are not directly correlated with annual precipitation amount (Fig. 5.8), contrary to the negative relationship that has been reported elsewhere (Loader and Hemming, 2004). In addition, a positive relationship has been observed previously between the $\delta^{18}O$ values of cellulose and annual precipitation amount (Saurer et al., 1997). Because cellulose is a principal component of pollen, a positive correlation between pollen $\delta^{18}O$ values and annual precipitation amount might also be expected. Its absence suggests that annual precipitation amount is not a major direct control on pollen $\delta^{18}O$ values. As discussed further below, however, it can affect the water sources utilized by some species, which can impart a distinctive $\delta^{18}O$ signature.

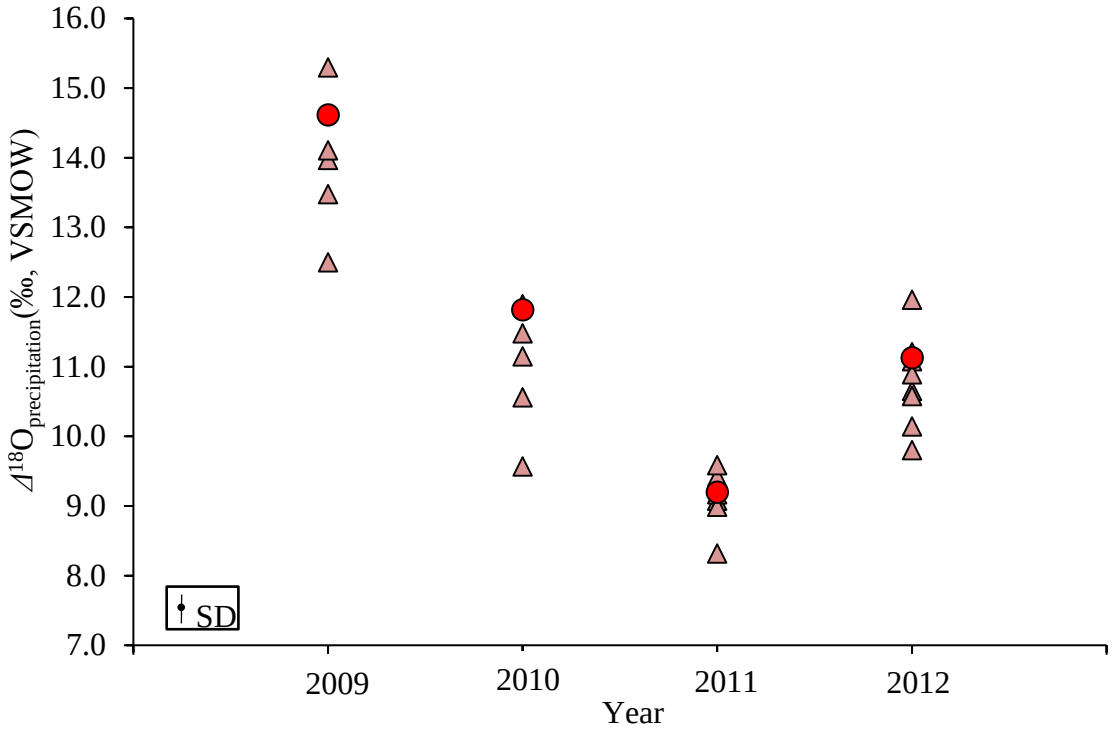

Figure 5.6 $\Delta^{18}O_{precipitation}$ for pollen of *P. resinosa* from a single tree (red circles) compared to the $\Delta^{18}O_{precipitation}$ for pollen from all other trees of *P. resinosa* (pink triangles), based on precipitation $\delta^{18}O$ values for October/November of the previous growing season.

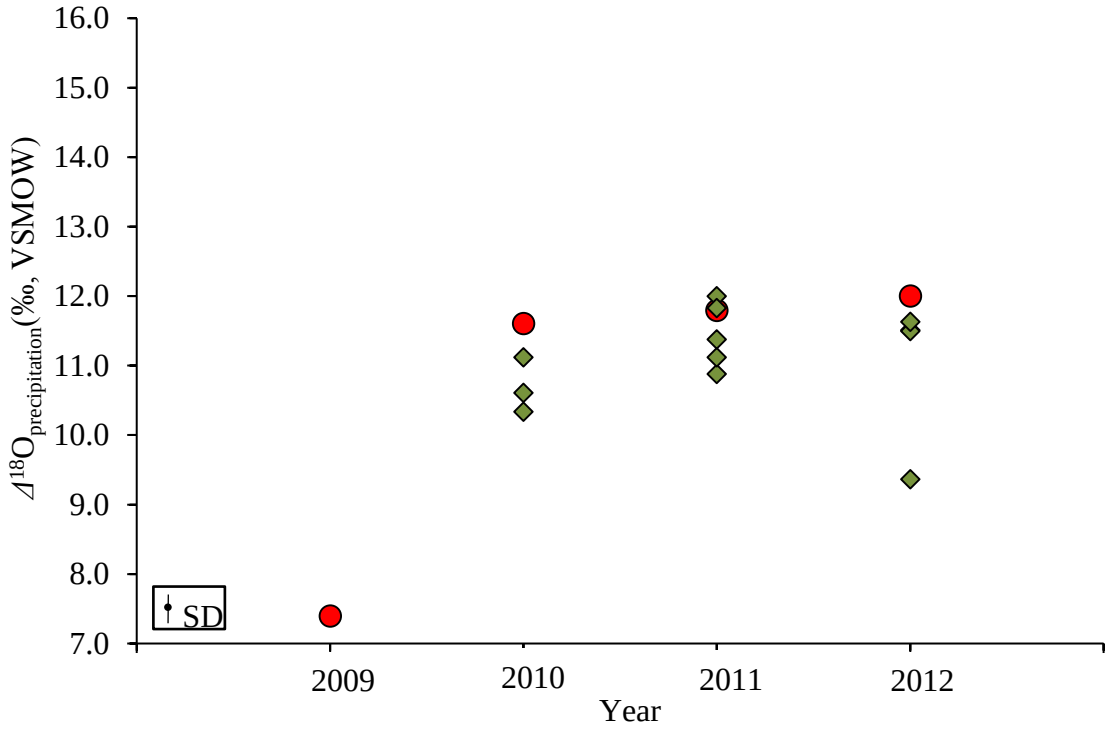

Figure 5.7 $\Delta^{18}O_{precipitation}$ for pollen of *P. strobus* from a single tree (red circles) compared to the $\Delta^{18}O_{precipitation}$ for pollen from all other trees of *P. strobus* (green diamonds), based on precipitation $\delta^{18}O$ values for October/November of the previous growing season.

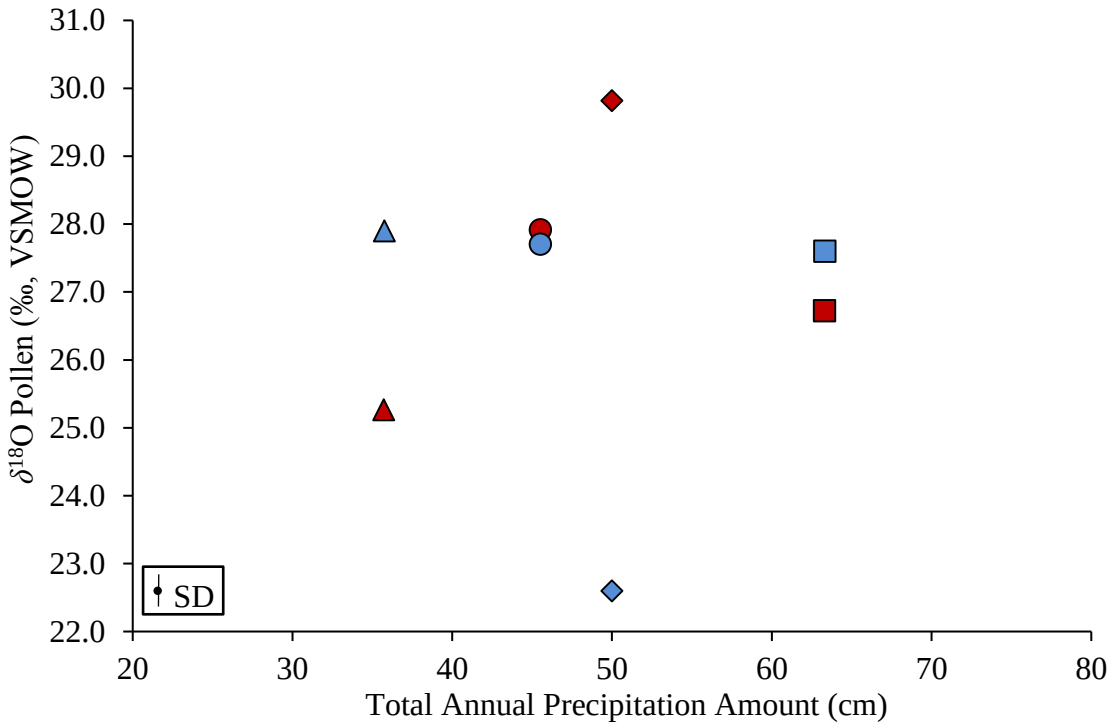

Figure 5.8 The δ^{18}O values of Pinery pollen from a single tree of *P. resinosa* (red) and *P. strobus* (blue) for 2009 (diamonds), 2010 (circles), 2011 (triangles), and 2012 (squares) compared with precipitation amount for the previous growing season.

5.1.6 Oxygen isotope compositions of pollen among plant species at the Pinery: additional considerations

As discussed earlier, the lower $\delta^{18}O$ values of *T. latifolia* pollen relative to other species examined in this study reflects its status as an obligate wetland species that grows in water ~0.5 to 1 m in depth. As such, transpiration has a very limited effect on plant water and hence the $\delta^{18}O$ values of sugars used in its pollen formation. The $\Delta^{18}O_{precipation}$ values of grass pollen at the Pinery are higher than those of *T. latifolia* because a transpiration signal is more strongly preserved by sugars utilized for grass pollen formation. As explained earlier, this signature is even more strongly developed in sugars used to form tree pollen.

Among *P. resinosa*, *P. strobus*, and *Q. rubra*, species-specific processes that produce significantly different mean pollen $\delta^{18}O$ values in some years but not in others are difficult to identify, given that micro-environmental conditions are much the same for all species. Conceivably, variations in stomatal conductance could produce these differences. Species with higher stomatal conductance undergo greater transpiration (Farquhar and Sharkey, 1982), resulting in higher $\delta^{18}O$ values of leaf tissues. During periods of moderate water stress, plants respond by increasing the number of stomata (Zhang et al., 2006), but decreasing stomatal size (Spence et al., 1986), in an effort to maximize water use efficiency. Broad-leaf trees such as *Q. rubra* have more stomata than conifers such as *P. resinosa* and *P. strobus* (Wang et al., 2015) and hence should undergo greater transpiration during drier periods, such as in 2010 at the Pinery. Such a change is not reflected in pollen $\delta^{18}O$ values (Table 5.1), however, suggesting that other factors affect pollen $\delta^{18}O$ values more strongly.

As discussed earlier, a change in water sources is a more likely candidate. The main tap-root of *P. resinosa* can reach depths of up to 5 m (Brown and Lacate, 1961), whereas *P. strobus* and *Q. rubra* usually have a more lateral root system with no main tap root. The water table at the Pinery is located at ~5-6 m below the forest surface; *P. resinosa* therefore can access groundwater directly. If *P. resinosa* uses groundwater during times

of water stress, its pollen $\delta^{18}O$ values should be lower than the other tree species, as observed for pollen in 2011 following the drier conditions of 2010 (Table 5.1).

5.1.7 Oxygen isotope composition of cellulose and its relationship to pollen at the Pinery

The $\delta^{18}O$ values for cellulose from leaves and stems of the grass species differ by less than 1‰; however, these values are an average of 5‰ higher than their respective pollen $\delta^{18}O$ values for both species (Fig. 4.2, Table 4.6). Grass leaf and stem cellulose collected at the same time as pollen likely formed using sugars produced during the same growing season. The $\delta^{18}O$ values of the cellulose of leaves and stems are expected to be higher than pollen $\delta^{18}O$ values, because they do not follow the same biological pathways subsequent to synthesis. Grass leaf water undergoes transpiration, which increases its $\delta^{18}O$ values, producing sugars enriched in ^{18}O relative to water taken in at the root. Sugars used in cellulose production remain in the leaf, thus retaining their high $\delta^{18}O$ values. Sugars used in pollen synthesis are translocated via the phloem downward along the stem, where continuous loading and unloading of photosynthates allows for exchange with non-transpired water, which is more depleted of ^{18}O. The slightly lower $\delta^{18}O$ values observed in grass stems relative to leaves is expected because there is likely at least some small amount of exchange with source water within the stem. Similar to the grasses, the $\delta^{18}O$ values of leaves and stems of *T. latifolia* are higher than average bulk pollen $\delta^{18}O$ values by 5.3‰ (leaves) and 6.3‰ (stems). As with the grasses, this is expected because of the differential translocation pathway of sugars subsequent to synthesis.

The $\delta^{18}O$ values of cellulose for leaves and stems of *Q. rubra* do not show as large a difference from pollen $\delta^{18}O$ values as do grass species. Leaf cellulose $\delta^{18}O$ values are an average of 2.1‰ higher than pollen $\delta^{18}O$ values, and stem cellulose $\delta^{18}O$ values are an average of 0.6‰ lower than pollen $\delta^{18}O$ values. This is expected because leaves and stems of *Q. rubra* are formed at the same time as pollen synthesis. Sugars used in the

production of leaves, stems, and pollen are produced in the previous growing season, and stored in all perennial parts of the tree over the winter as an energy source for initial spring growth (Loescher et al., 1990). When remobilized, sugars do not travel long distances during translocation in the phloem, and therefore retain similar $\delta^{18}O$ values. The oxygen isotope composition of *Q. rubra* leaves sampled early in the growing season (May) is much lower (4.3‰) than leaves sampled late in the season (October) (Fig. 4.3). This difference is not observed in *P. resinosa* and *P. strobus*, because they retain their needles for up to three years, and when sampling it is unknown from which year needles formed. In *Q. rubra*, leaves sampled late in the growing season have a greater surface area. More stomata result in increased rates of transpiration, and therefore higher $\delta^{18}O$ values. The low $\delta^{18}O_{stem}$ relative to $\delta^{18}O_{leaf}$ values in *Q. rubra* is also likely a result of higher rates of transpiration in the leaf relative to stem.

The oxygen isotope composition of needle cellulose and pollen from the coniferous tree species varies considerably. In *P. resinosa*, average pollen $\delta^{18}O$ values are 3.4‰ lower than the average cellulose $\delta^{18}O$ value of needles, whereas the average difference is 1.1‰ for *P. strobus*. The coniferous *P. resinosa* and *P. strobus* retain their needles for an average of three and two years, respectively (Ewers and Schmid, 1981), and needle loss does not occur simultaneously. As such, the $\delta^{18}O$ values of cellulose from the analysis of multiple needles is not representative of a particular growing season, and provides a reasonable explanation for the varying differences between cellulose and pollen $\delta^{18}O$ values in *P. resinosa* versus *P. strobus*.

5.1.8 Oxygen isotope composition of tree pollen from North American sites

The precipitation $\delta^{18}O$ values calculated using *Quercus* (Oak) and *Acer* (Maple) pollen by applying the cellulose-water oxygen isotope fractionation factor are all much higher than those expected for precipitation during October/November of the previous growing

season (Table 5.2, 2011; Table 5.3, 2012). This difference likely arises both from transpiration and near surface soil-water evaporation. The extent of ^{18}O-enrichment is consistent with the amount of evapotranspiration at a given location. Locations having generally lower annual evapotranspiration rates (Tables 5.2, 5.3; Fig. 5.10; Mu et al., 2011) have larger differences between predicted $\delta^{18}O_{precipitation}$ and measured $\delta^{18}O_{precipitation}$ values. Indeed, the difference in calculated $\delta^{18}O_{precipitation}$ (based on Bowen and Revenaugh, 2003) and measured $\delta^{18}O_{precipitation}$ versus transpiration rate shows a negative relationship (Fig. 5.11). This emphasizes the strong link between relative humidity and pollen δ^{18}O values, in this instance at the scale of a continental landscape.

5.2 Carbon isotopes and C/N ratios

5.2.1 C/N ratios of pollen

The average C:N ratios of coniferous species (*P. resinosa* = 26, *P. strobus* = 25) and the deciduous *Q. rubra* (C:N = 9) are consistent with those reported by Descolas-Gros and Schölzel (2007). *Ammophila breviligulata* and *A. gerardii* have C:N ratios of 14 and 15 respectively, and *T. latifolia* a ratio of 18 (Appendix 1). Kristensen et al. (2004) suggest that the lower C:N ratio observed in deciduous species is a result of increased nitrogen leaching compared to coniferous species. The differences reported in C:N ratios of tree, grass, and marsh species in this study may be a result of variable degrees of nitrogen leaching in respective soil systems; however, a larger sampling size and soil sampling is necessary for further comment.

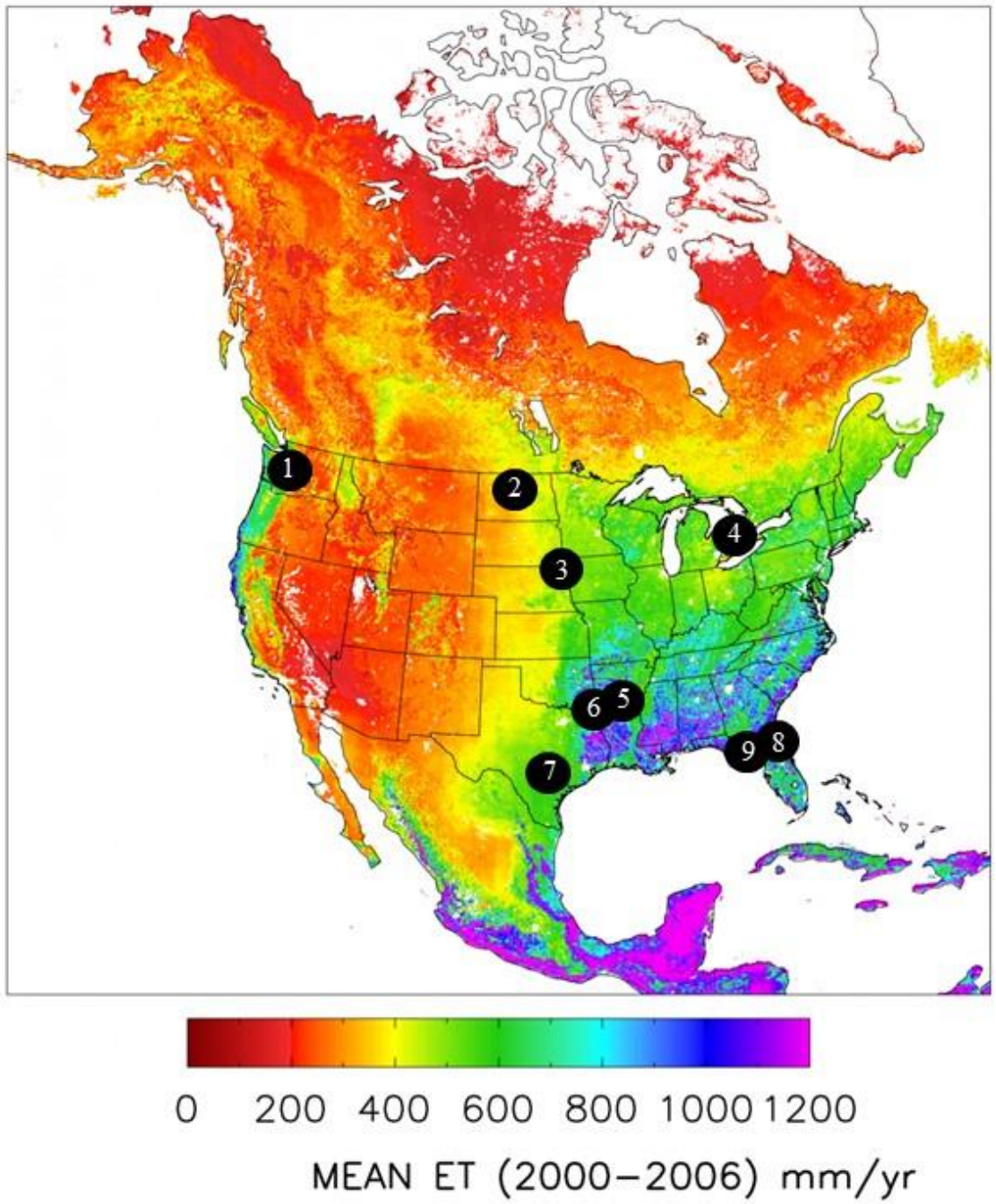

Figure 5.9 Map of North America showing mean evapotranspiration rates for 2000 to 2006. Numbers correspond to locations listed in Tables 5.2 and 5.3 (modified from Mu et al., 2011).

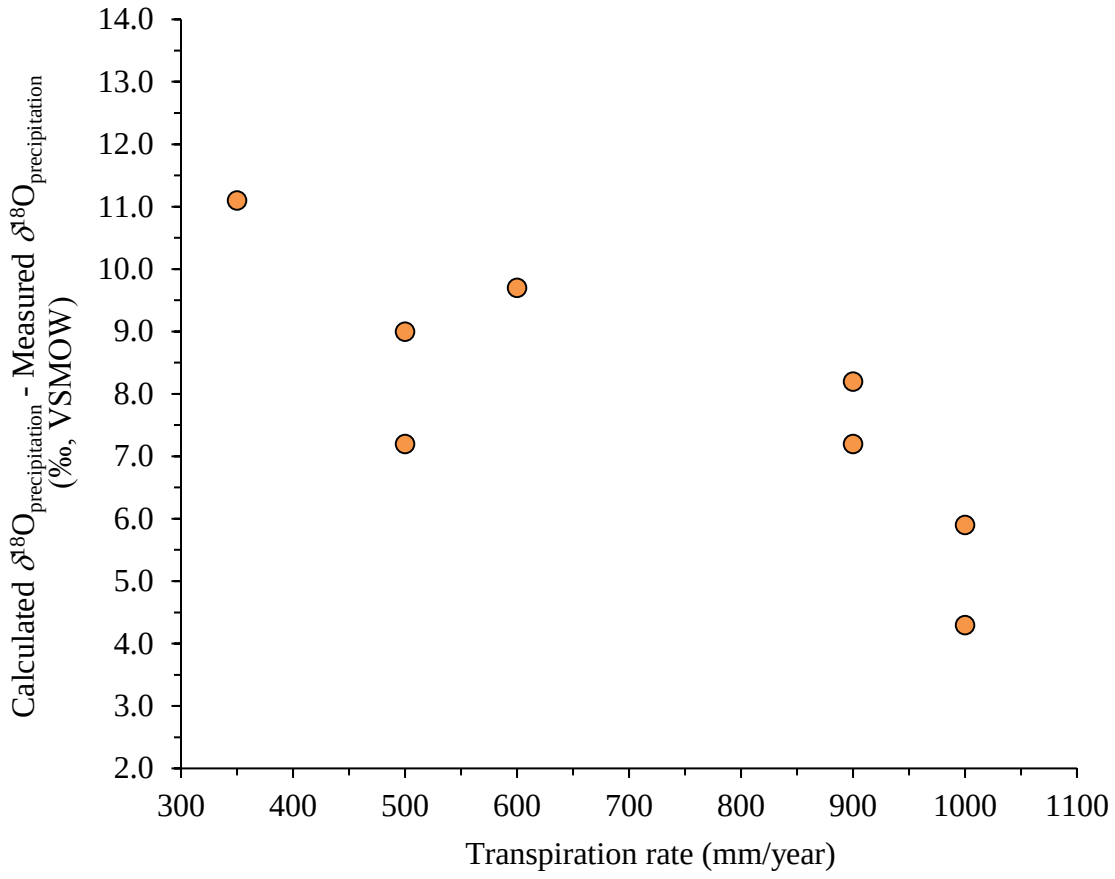

Figure 5.10 Calculated $\delta^{18}O_{precipitation}$ – measured $\delta^{18}O_{precipitation}$ versus transpiration rate for North American sites.

Table 5.2 $\delta^{18}O$ values of pollen from North American sites in 2011 compared to the transpiration rate and the oxygen isotope compositions of measured and predicted annual precipitation amount.

Site Number	Location	$\delta^{18}O$ of Precipitation (‰)[1]	Measured $\delta^{18}O$ Pollen (‰)	Predicted $\delta^{18}O$ of Precipitation (‰)[2]	Predicted minus measured Precipitation $\delta^{18}O$ (‰)[3]	Transpiration rate (mm/year) from 2000-2006[4]
2	Minot, ND	–16.5	+21.6	–5.4	+11.1	300-400
3	Vermillion, SD	–12.2	+23.8	–3.2	+9.0	400-600
4	The Pinery, ON	–7.9*	+26.3	–0.7	+7.2	400-600
5	Hot Springs, AR	–6.5	+28.7	+1.7	+8.2	800-1000
6	Arkadelphia, AR	–6.3	+27.9	+0.9	+7.2	800-1000
7	College Station, TX	–4.8	+31.9	+4.9	+9.7	600
8	Madison, FL	–5.1	+27.8	+0.8	+5.9	900-1100
9	Monticello, FL	–5.1	+26.2	–0.8	+4.3	900-1100

[1] Annual $\delta^{18}O$ precipitation at that site determined following Bowen and Revenaugh (2003).
[2] Pollen $\delta^{18}O$ values minus 27‰ (cellulose-water fractionation factor).
[3] Difference between precipitation $\delta^{18}O$ calculated from pollen and annual precipitation $\delta^{18}O$ values determined following Bowen and Revenaugh (2003).
[4] Transpiration rate (Mu et al., 2011).
* Measured average $\delta^{18}O$ value for the growing season.

Table 5.3 $\delta^{18}O$ values of pollen from North American sites in 2012 compared to the transpiration rate and the oxygen isotope compositions of measured and predicted annual precipitation amount.

Site Number	Location	$\delta^{18}O$ of Precipitation (‰)[1]	Measured $\delta^{18}O$ Pollen (‰)	Predicted $\delta^{18}O$ of Precipitation (‰)[2]	Predicted minus measured Precipitation $\delta^{18}O$ (‰)[3]	Transpiration rate (mm/year) from 2000-2006[4]
1	Seattle, WA	–10.8	+23.0	–4.0	+14.8	200-600
4	The Pinery, ON	–7.9*	+25.7	–1.3	+6.6	400-600
5	Hot Springs, AR	–6.5	+29.9	+2.9	+9.4	800-1000
6	Arkadelphia, AR	–6.3	+30.2	+3.2	+9.5	800-1000
7	College Station, TX	–4.8	+27.3	+0.3	+5.1	600
8	Madison, FL	–5.1	+27.5	+0.5	+5.6	900-1100
9	Monticello, FL	–5.1	+28.4	+1.4	+6.5	900-1100

[1] Annual $\delta^{18}O$ precipitation at that site determined following Bowen and Revenaugh (2003).
[2] Pollen $\delta^{18}O$ values minus 27‰ (cellulose-water fractionation factor).
[3] Difference between precipitation $\delta^{18}O$ calculated from pollen and annual precipitation $\delta^{18}O$ values determined following Bowen and Revenaugh (2003).
[4] Transpiration rate (Mu et al., 2011).
* Measured average $\delta^{18}O$ value for the growing season.

5.2.2 Carbon isotope composition of pollen from the Pinery, 2009-2012

The pollen $\delta^{13}C$ values of C_3 plants in this study (–30.3 to –24.9‰) are typical of this photosynthetic pathway. The pollen $\delta^{13}C$ values of the C_4 grass *A. gerardii* (–12.9 to –11.3‰) are likewise typical of C_4 plants. As such, the carbon isotope composition of pollen from Pinery vegetation is a reliable indicator of C_3 versus C_4 photosynthetic pathway. There are no significant inter-annual differences in the carbon isotope composition of the C_3 pollen analyzed in this study for all species collectively (Table 4.4b) and the variability in $\delta^{13}C$ values amongst years is similar (Fig. 4.4).

The $\delta^{13}C$ values of plant tissue are also dependent on water availability (Kohn, 2010) and relative humidity (Nelson, 2012). Its carbon isotope composition should reflect these environmental conditions at the time of sugar synthesis (which for pollen from *P. resinosa*, *P. strobus*, and *Q. rubra*, is at the end of the previous growing season, as discussed earlier). During periods of moderate to severe drought, the $\delta^{13}C$ values of plant tissues increase because of stomatal closure to inhibit water loss. As a result, CO_2 availability decreases, lowering photosynthetic activity (Kumar and Singh, 2009). If water availability is the controlling factor on the carbon isotope composition of pollen, $\delta^{13}C$ values should increase during periods of water stress. During 2010, the amount of precipitation during the growing season was only half (35 cm) of that typical of other years (i.e., 63 cm in 2011). There are no trends in the $\delta^{13}C$ values of pollen, however, that reflect ^{13}C-enrichment arising from this period of decreased precipitation, either for grasses in 2010 or for trees in 2011. This level of water stress appears not to have been sufficiently severe to affect pollen $\delta^{13}C$ values.

When relative humidity is low, stomatal conductance decreases, limiting CO_2 uptake and thus increasing $\delta^{13}C$ values within tissues. Loader and Hemming (2004) suggest that the $\delta^{13}C$ values of pollen released by *Pinus sylvestris* reflect the period 4 to 6 weeks prior to pollen fly. In their study, pollen fly for *P. sylvestris* occurs between May and July.

Photosynthesis has been initiated for the growing season by the time *P. sylvestris* synthesizes pollen, and hence pollen formation does not utilize stored sugars from the previous growing season. In the present study, the carbon isotope compositions of tree pollen from *P. resinosa*, *P. strobus* and *Q. rubra* are not significantly correlated to relative humidity from either the previous growing season or (not unexpectedly) the 4 to 6 week period prior to pollen formation. The differences in relative humidity were not large enough to translate into significant differences in pollen $\delta^{13}C$ values.

The oxygen isotope compositions of pollen from *T. latifolia*, *A. breviligulata*, and *A. gerardii* suggest that sugars used in pollen synthesis formed 4-8 weeks prior to pollen fly. As with tree species, however, the $\delta^{13}C$ values of pollen of these species also show no significant correlation with relative humidity during this period.

5.2.3 Carbon isotope composition of pollen within plant species at the Pinery

The $\delta^{13}C$ values within individual species vary within years (Fig. 4.4). For example, the variation of *P. resinosa* and *P. strobus* in 2012 is 0.4‰. These small differences may be related to amount of irradiance and associated changes in temperature. Irradiance has been previously positively correlated to the $\delta^{13}C$ values of cellulose (Israeli et al., 1996). In this study, *P. resinosa* trees located beside graveled parking lots consistently yielded slightly higher pollen $\delta^{13}C$ values compared to trees located in more densely forested areas, which received less sunlight, as was also the case for *P. strobus*. All samples of *T. latifolia* have similar access to sunlight, and show slightly less variability in pollen $\delta^{13}C$ values from year to year (0.2 to 0.9‰ Table 5.1). Pollen from all samples of *Q. rubra*, however, was collected from locations with very similar access to sunlight from year to year, but showed similar variability in pollen $\delta^{13}C$ values as the conifers (0.3 to 1.4‰; Table 5.1). Only one sample of each grass species was analyzed each year, therefore no

further comment can be made regarding the effect of sunlight accessibility on carbon isotope composition for *A. breviligulata* and *A. gerardii* within a single year.

5.2.4 Differences in carbon isotope composition of pollen among plant species at the Pinery

The mean $\delta^{13}C$ values of pollen show less variation among species than pollen $\delta^{18}O$ values. For all years, pollen $\delta^{13}C$ values of *T. latifolia* are amongst the lowest compared to other species (Figure 4.4). This is likely because the roots and lower stems of *T. latifolia* are continuously immersed in water. Hence, the cattails are not affected by water stress, thus favouring lower $\delta^{13}C$ values.

Amongst tree species, when significant differences in the $\delta^{13}C$ values of pollen are present (Table 4.8), *P. resinosa* has the lowest mean $\delta^{13}C$ values (Table 5.1). This is expected if water stress is the major factor responsible for the differences in $\delta^{13}C$ values amongst tree species. *Pinus resinosa* has a deeper root system than *P. strobus* and *Q. rubra*, enabling the former to reach water at greater depths during times of water stress. As has been discussed earlier, the amount of precipitation in the 2010 growing season was approximately half that of 2011. If this difference was sufficient to cause water stress, then the $\delta^{13}C$ values of *P. strobus* and *Q. rubra* pollen collected in 2011 should be higher than *P. resinosa* pollen. This disparity should be smaller for tree pollen collected in 2012, as it reflects sugar formation during a year (2011) of lower water stress. There is little difference, however, in pollen $\delta^{13}C$ values between *P. resinosa* and other tree species in 2011 and in 2012. This suggests that microclimatic variations in water availability, relative humidity and temperature, as well as genetic differences among species, are insufficient to cause significant differences in tree pollen carbon isotopic compositions across the Pinery.

5.2.5 Carbon versus oxygen isotope composition of pollen among plant species at the Pinery

There are positive correlations between the carbon and oxygen isotope compositions of *P. resinosa*, $r(28) = 0.69$, $p < 0.01$, *P. strobus*, $r(16) = 0.46$, $p < 0.01$, and *T. latifolia*, $r(10) = 0.63$, $p < 0.05$ (Fig. 5.12). Such correlations are expected because both oxygen and carbon isotopes commonly show similar directional changes in $\delta^{18}O$ and $\delta^{13}C$ values in response to temperature shifts (Szymczak et al., 2011). When temperature increases, the $\delta^{18}O$ value of precipitation also generally increases, which in turn is reflected in plant water and plant tissues. Likewise, relative humidity affects the $\delta^{18}O$ value of leaf water, and therefore plant tissues. When aridity is high (low relative humidity), stomatal openings close, forcing the plant to use the ^{18}O-rich water remaining in the cell cytoplasm. Closure of stomata also prevents access to ^{12}C-rich atmospheric CO_2. As a result, low relative humidity results in higher $\delta^{18}O$ and $\delta^{13}C$ values of plant tissues.

The $\delta^{18}O$ and $\delta^{13}C$ values of *Q. rubra* are negatively correlated, $r(15) = -0.55$, $p < 0.05$ (Fig. 5.11c). The reason for this decoupling of oxygen and carbon isotope pollen compositions is not known. There is no significant correlation between oxygen and carbon isotope compositions of pollen from *A. breviligulata* and from *A. gerardii*, but the data available for these grasses are too sparse at the present time to discuss this observation further.

5.2.6 Carbon isotope composition of cellulose and its relationship to pollen at the Pinery

The carbon isotope compositions of leaf and stem cellulose for non-coniferous plants in this study (*Q. rubra*, *T. latifolia*, *A. breviligulata*, and *A. gerardii*) differ from bulk pollen $\delta^{13}C$ values by a maximum of ±3‰, similar to results reported by Jahren (2004) for a variety of vascular plants. The $\delta^{13}C$ values of leaf and stem cellulose of the Pinery

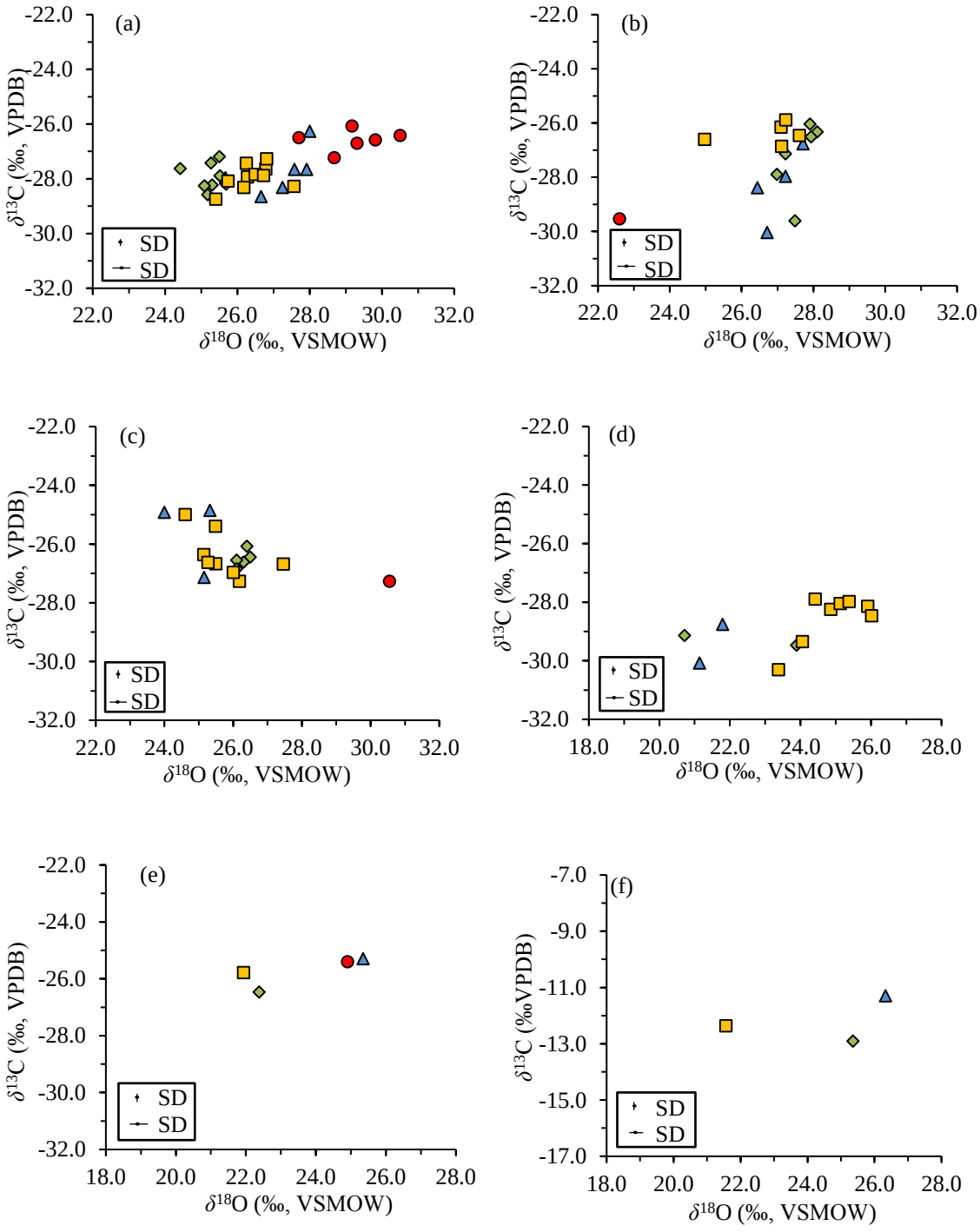

Figure 5.11 Carbon versus oxygen isotope compositions for pollen of (a) *P. resinosa*, (b) *P. strobus*, (c) *Q. rubra*, (d) *T. latifolia*, (e) *A. breviligulata*, and (f) *A. gerardii*, sampled in 2009 (red circles), 2010 (blue triangles), 2011 (green diamonds), and 2012 (yellow squares).

conifers (*P. resinosa* and *P. strobus*, Fig. 4.7) were not different from each other, and were not significantly different from bulk pollen δ^{13}C values. This may be because needles remain for a period of up to three years, thus reducing the contrast with pollen δ^{13}C values for any particular year. This suggests that the carbon isotope composition of pollen from coniferous species such as these can be used as a proxy for cellulose δ^{13}C values of foliage and stem material.

In the green-stemmed, deciduous species *Q. rubra*, the δ^{13}C values of leaf and stem cellulose (Fig. 4.7) were not significantly different from each other, notwithstanding a greater difference between measured compositions relative to woody-stemmed species. Both leaf and stem cellulose δ^{13}C values were lower than pollen δ^{13}C values, similar to results for green-stemmed species reported by Jahren (2004). The lower leaf and stem cellulose δ^{13}C values in green-stemmed plants can be explained by their higher concentration of lignins and lipids, both of which are depleted of ^{13}C relative to bulk tissue by up to 4‰ (Benner et al., 1987) and 5-10‰ (Chikaraishi et al., 2003), respectively (Jahren, 2004). The leaves of *Q. rubra* are broad, and have a larger surface area than the needles of *P. resinosa* and *P. strobus*, therefore they contain more epicuticular waxes. These waxes are composed primarily of lipids, which contribute to the lower δ^{13}C values of *Q. rubra* leaves. In addition, stems of *Q. rubra*, unlike conifer stems, are able to photosynthesize, and are also composed of lipid-rich waxes, which can account for their lower δ^{13}C values.

The carbon isotope compositions of leaf cellulose from *T. latifolia*, and the grass species *A. breviligulata* and *A. gerardii* (Fig. 4.6), are not significantly different from their respective stem cellulose δ^{13}C values. All three species, however, have pollen δ^{13}C values that are slightly lower (*T. latifolia*: 2.2‰ lower, *A. breviligulata*: 0.7‰ lower, and *A. gerardii*: 0.8‰ lower) than leaf and stem cellulose δ^{13}C values. The lower pollen δ^{13}C values are likely a result of the lower lipid and higher cellulose contents in the leaves and stems of grasses (Chesworth et al., 1998) and *T. latifolia* (Duke, 1983), compared to tree species. Larger amounts of cellulose, which can have δ^{13}C values up to 4‰ higher than

bulk plant tissue (Marino and McElroy, 1991), and lower quantities of lipids results in higher leaf and stem $\delta^{13}C$ values compared to bulk pollen $\delta^{13}C$ values.

5.2.7 Carbon isotope composition of pollen at sites across North America

Previous studies report a positive relationship between plant tissue such as cellulose and relative humidity (e.g., Barbour and Farquhar, 2000). Based on these earlier reports, we expect Minot, ND (lowest annual relative humidity, 58%) to yield the lowest pollen $\delta^{13}C$ values, and the Pinery, ON (highest annual relative humidity, 77%) the highest. As reported earlier (Fig. 4.10), no significant correlation was observed among these sites. It is very likely that too few sites were examined on this scale to properly test for a relationship between pollen $\delta^{13}C$ values and relative humidy, and that the spread in relative humidity values among these sites is too small for patterns of variation to emerge.

5.3 Nitrogen isotopes

5.3.1 Nitrogen isotope composition of pollen from the Pinery, 2009-2012

In the Pinery, tree species (*P. resinosa*, *P. strobus*, and *Q. rubra*) have similar pollen $\delta^{15}N$ values (–7.2 to –1.7‰). The C_4 grass *A. gerardii* has similar pollen $\delta^{15}N$ values to the tree species (–3.1 to –1.8‰). By comparison, the C_3 grass *A. breviligulata* has higher pollen $\delta^{15}N$ values (+0.9 to +3.5‰), and still higher pollen $\delta^{15}N$ values were measured for *T. latifolia* (+5.9 to +11.4 (Fig. 4.8). These variations reflect both variations in the source(s) of nitrogen used by each species and the processes that fractionate the $\delta^{15}N$ values of bioavailable nitrogen.

The nitrogen isotope composition of plant tissue is largely determined by soil $\delta^{15}N$ values, which themselves show significant variation on the micro-environmental scale (Högberg, 1997). Nitrogen is available to plants through organic matter in the soil or – in some cases – from the atmosphere. In the older, forested dunes of the Pinery, there is up to three times as much NH_4^+ and NO_3^- in the soil compared to younger, open sand dunes (Maun, 2009). This increase in soil nitrogen availability in mature dunes is a result of higher species diversity, increased amounts of soil organic matter, and greater stabilization of plant communities. In the soils inhabited by *A. breviligulata* on the open, young dunes, there is little organic matter, and thus little accessible nitrogen. Conversely, *A. gerardii* was collected at or near the same locations as tree species in the Pinery, on older dunes away from the shore, where soil has accumulated, and there is a greater supply of nitrogen in soil organic matter. The $\delta^{15}N$ values of *A. breviligulata* are significantly higher than those of *A. gerardii* (Fig. 4.8). *Ammophila brevilugulata* obtains some nitrogen via the diazotrophic bacteria *Azotobacter*, which fixes atmospheric nitrogen into usable ammonium ions (Maun, 2009). There may also be aquatic nitrogen input. McCormick (2008) report that $\delta^{15}N$ values of bulk stems of *A. breviligulata* along Lake Michigan were higher at the shoreline compared to stems measured progressively away from the shore, which was attributed to a 23% contribution from aquatic nitrogen.

The nitrogen isotope composition of *A. breviligulata* pollen increases slightly each year from 2009 to 2012 (+0.9 to +3.5‰). The precise timing for formation of nitrogen-bearing protein molecules in pollen is unknown. Maricle et al. (2011) report increasing $\delta^{15}N$ values in bulk leaf material with increasing leaf temperature and transpiration, and Szpak et al. (2013) report decreasing $\delta^{15}N$ values with precipitation amount. Precipitation amount is likely not the cause of increasing $\delta^{15}N$ values in *A. breviligulata*, because total precipitation for the months shortly before and during pollen synthesis (March to July) does not show a pattern of increase between 2009 and 2012 (338 mm in 2009, 340 mm in 2010, 392 mm in 2011, and 131 mm in 2012). There is a weak pattern of temperature increase (March to July), from 2009 to 2012 (15˚C in 2009, 17˚C in 2010,

16˚C in 2011, and 20˚C in 2012). A larger sample size and sampling period, however, is necessary to explore these putative relationships further.

It is unlikely that rooting depth has had a noticeable effect on the pollen δ^{15}N values of species examined in this study that grow on more mature Pinery soils. For example, the pollen δ^{15}N values of *A. gerardii* are similar to those of tree species; however, their root system is very different. While roots of established *A. gerardii* may reach depths of up to 2.4 m (Weaver, 1968), roots of trees such as *P. resinosa* may penetrate to more than twice that depth.

The nitrogen isotope composition of *T. latifolia* is significantly higher than all other plant types. Average pollen δ^{15}N values from *T. latifolia* plants in this study (+8.0‰) are similar to those reported by Cloern et al. (2002) for bulk leaf material from cattails (+7.0‰). Marshes act as sinks for nutrients such as nitrogen, which translates into higher δ^{15}N values. *Typha latifolia* is constantly submerged in water; as such, it is likely influenced by benthic denitrification, which facilitates formation and assimilation of ^{15}N-rich NO_3^- by cattail roots (Hall et al., 2015).

Although denitrification in the aquatic environment may account for the higher δ^{15}N values in *T. latifolia* relative to other non-marsh species, there is also a difference in pollen δ^{15}N values between sampling sites; *T. latifolia* pollen sampled in Zurich has consistently higher δ^{15}N values (+9.8 to +11.4‰) than in London (+5.9 to +9.2‰). In agricultural regions such as Zurich, Ontario, the nitrogen isotope composition of watersheds is expected to be higher than non-agricultural areas such as the sampling site in London (Peipoch et al., 2012), as a result of greater nitrogen losses through denitrification, ammonia volatilization and the addition of ^{15}N-rich nitrogen from manure and septic effluents (Hall et al., 2015).

5.3.2 Nitrogen isotope composition of pollen within plant species

There is significant variation in pollen δ^{15}N values within species; for example, *P. resinosa* and *Q. rubra* exhibit 3.9‰ and 3.1‰ differences between highest and lowest δ^{15}N values in 2012 (Fig. 4.8). These variations are likely not a result of differences in temperature or transpiration among tree locations. All trees from which pollen was collected and sampled for *Q. rubra* were located relatively close to each other, and had almost identical air flow and access to sunlight. By default, it appears that these differences arise from variations in the processes by which fractionation has affected the isotopic composition of bioavailable nitrogen. A more open versus a more closed nitrogen cycle at any particular location can produce such variation (Tahmasebi, 2015) but the data presently available for the Pinery are insufficient to test this idea further.

5.3.3 Nitrogen isotope composition of tree pollen across North America

The variations in pollen δ^{15}N values from *Quercus* (Oak) and *Acer* (Maple) from the eight sites across the USA, and the Pinery, show no specific relationship with annual precipitation amount during the same year as pollen collection (Fig. 5.12) or annual temperature (Fig. 5.13), notwithstanding earlier studies that suggest such a relationship (Kang et al., 2011; Amundson et al., 2003). The handful of sites examined in the present study is almost certainly far too few for such a relationship to be detected.

There is also no apparent negative correlation between pollen δ^{15}N values and altitude, unlike that reported by Szpak et al. (2013) for the Andes. The difference in elevation (<400 m) among the handful of sites examined in the current study, however, is likely too small for a pattern to be detected, even if elevation was a dominant factor affecting pollen δ^{15}N values over a larger gradient.

Another reason for the measured spread of tree pollen δ^{15}N values could be variations in mycorrhizal associations (Hobbie et al., 1999, 2005; Trudell et al., 2004; Fodor et al., 2011), but no data are available to test this idea for the individual sites. More broadly, there are no data for these sites concerning nitrogen availability in their soils. Nitrogen availability is affected by temperature, aridity, soil erosion and fertilizer addition, among other factors. These factors affect processes such as denitrification, which in turn affects soil δ^{15}N values. The δ^{15}N values of plant tissues are known to be positively correlated to nitrogen availability in the soil (Garten and van Miegroet, 1993), which in turn is related to the open versus closed character of the nitrogen cycle, which often varies with aridity and temperature (Craine et al, 2009; Tahmasebi, 2015).

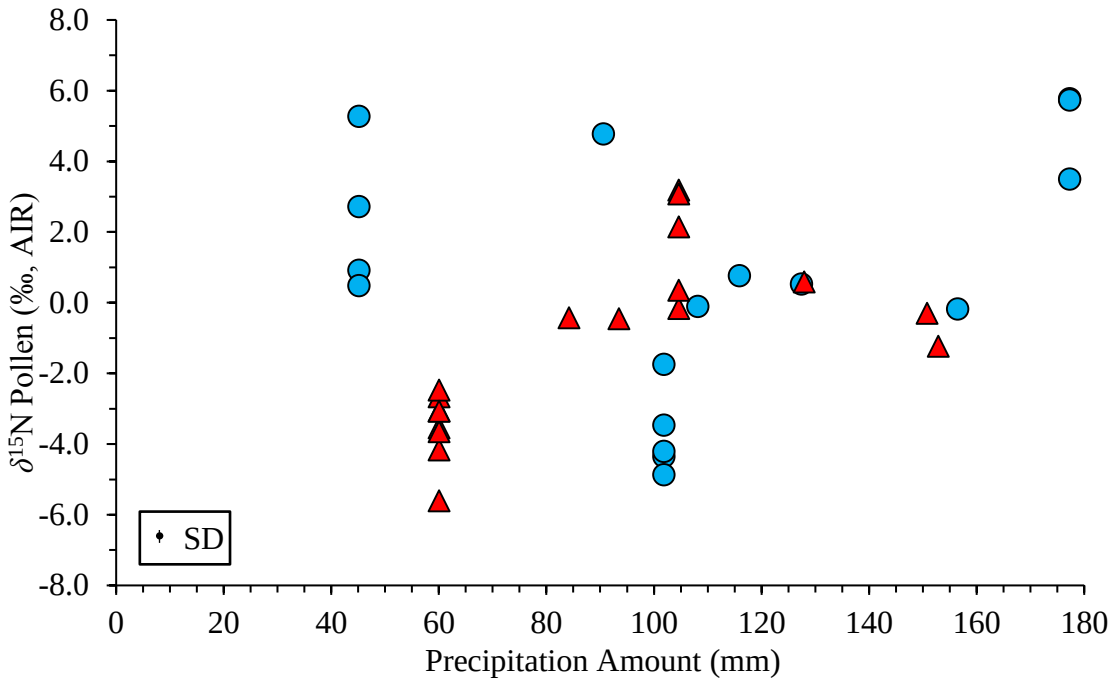

Figure 5.12 Nitrogen isotope composition for tree pollen versus precipitation amount (mm) in 2011 (blue circles) and 2012 (red triangles).

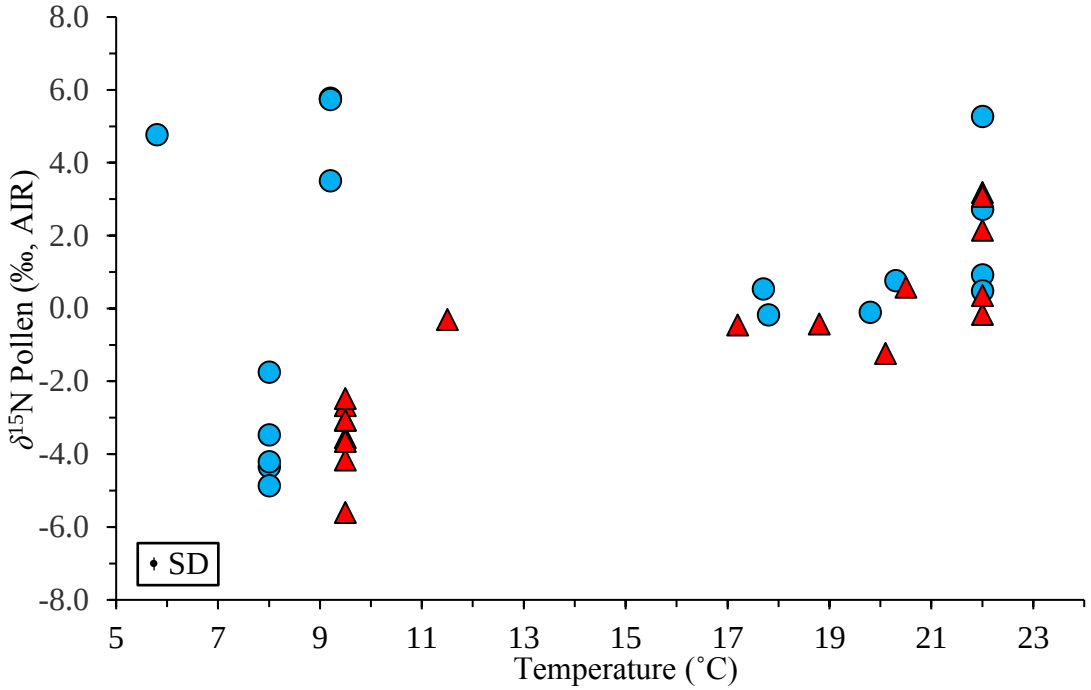

Figure 5.13 Nitrogen isotope composition for tree pollen versus average annual temperature in 2011 (blue circles) and 2012 (red triangles).

Chapter 6

6 Conclusions

This thesis has examined environmental factors affecting the stable oxygen, carbon, and nitrogen isotope compositions of pollen of C_3 (*P. resinosa*, *P. strobus*, *Q. rubra*, *A. breviligulata*, *T. latifolia*) and C_4 (*A. gerardii*,) plants from Pinery Provincial Park, Ontario. While previous research has sought to investigate the relationship between the carbon isotope composition of pollen and environment, that between the oxygen isotope composition of pollen and environmental conditions has been much less explored. This thesis has focused in particular on that relationship.

6.1 Major environmental influences affecting the oxygen, carbon, and nitrogen isotope compositions of pollen

The oxygen isotope composition of pollen is determined by multiple factors. Ultimately, the $\delta^{18}O$ value of precipitation determines the initial oxygen isotope composition of sugars used in pollen synthesis; however, the degree to which the $\delta^{18}O$ values of these sugars are changed following synthesis is determined by plant type and physiology. The pollen $\delta^{18}O$ value of the marsh-type plant *T. latifolia* is determined largely by the $\delta^{18}O$ values of precipitation at the time of sugar formation, with minor ^{18}O enrichment resulting from evaporation of the surface water in which the cattails are rooted. Because the only path available to sugars traveling from the leaf to the site of pollen synthesis is down to the root, and then up to the inflorescence, exchange with lower ^{18}O root (source) water substantially reduces any enrichment of ^{18}O in sugars that occurred because of transpiration in the leaf. Pollen from *T. latifolia* is therefore a good proxy for the oxygen isotope composition of precipitation at the time of sugar formation, at least in climatic regimes where relative humidity is sufficiently high to minimize ^{18}O enrichment of

surface water during extensive evaporation. This conclusion is also likely transferable to other plants that live rooted in marsh-like environments.

The oxygen isotope composition of pollen from the two grass species is more strongly affected by transpiration in the leaf than is the case for cattails. Located along the stem, sugars produced in the leaf, and acquiring ^{18}O-enriched signatures from transpired leaf water, may travel either to the stem and up to the site of pollen synthesis, or they may travel to the stem and down to the roots where (as with *T. latifolia*) they interact with typically lower ^{18}O soil water, before then travelling up to the inflorescence. Because some sugars do not undergo oxygen isotopic exchange by interaction with soil water, the oxygen isotope composition of grass pollen retains some of the ^{18}O enrichment signal acquired during transpiration in the leaf.

The δ^{18}O values of the tree pollen species sampled (*P. resinosa*, *P. strobus*, *Q. rubra*) is heavily influenced by leaf water ^{18}O-enrichment during transpiration. The oxygen used in pollen formation is derived from sugars produced in leaves during the previous growing season. These sugars are stored over the winter, and then remobilized to fuel initial spring growth. Because these sugars are stored throughout the entire tree, they do not necessarily travel down the trunk where they would undergo oxygen isotopic exchange with lower ^{18}O soil water. The sugar and hence pollen δ^{18}O values therefore largely retain the ^{18}O enrichment signal acquired during transpiration in the leaf. The amount of ^{18}O enrichment in tree pollen does not differ between the deciduous and conifer species examined in this thesis, further suggesting that, in addition to the amount of ^{18}O enrichment of leaf water used in sugar formation, the subsequent translocation pathway of sugars used in pollen formation is a predominant factor in determining the oxygen isotope composition of pollen. Studies planning to use the δ^{18}O values of grass and tree pollen for paleoclimatic interpretations of source water (i.e., precipitation) oxygen isotopic composition must consider the degree to which transpiration has modified these values, likely in response to relative humidity and temperature at the time of sugar formation.

Compared to pollen $\delta^{18}O$ values, the $\delta^{13}C$ values of C_3 plants varied very little among the species examined in this study. There was no significant correlation between pollen $\delta^{13}C$ values and either relative humidity or source water availability.

At the Pinery, the nitrogen isotope composition of pollen is likely determined by the $\delta^{15}N$ value of its source, regardless of species. This accounts for the similarity between the pollen $\delta^{15}N$ values of the grass species *A. gerardii* and the three tree species sampled from the Pinery, all of which were sampled from the same soils. The grass species, *A. breviligulata*, by comparison, had higher pollen $\delta^{15}N$ values, and was sampled from an active dune location containing very little soil organic matter. It likely relied on nitrogen sources (i.e., atmospheric nitrogen, aquatic nitrogen) that were different from the forest soil. The much higher pollen $\delta^{15}N$ values of the marsh species, *T. latifolia*, are likely the result of substantial denitrification, which enriches remaining nitrogen in ^{15}N, plus varying contributions from other ^{15}N-rich sources such as agricultural and septic effluents.

6.2 Factors controlling the variability in the isotopic composition of pollen within species, from the same location

The amount of variation in pollen $\delta^{18}O$ values within tree species is principally influenced by precipitation amount. For example, relative to other tree species, *Pinus resinosa* pollen had lower and less variable $\delta^{18}O$ values in years of sugar formation that had significantly less precipitation than other years. The roots of *P. resinosa* reach greater depths than *P. strobus* or *Q. rubra*, and are thus able to access groundwater, which at the Pinery is more depleted of ^{18}O and less variable in $\delta^{18}O$ values than late growing season precipitation or soil water. During years where precipitation amount is greater, more soil water is available at higher levels, which is known to have higher but

more variable $\delta^{18}O$ values as a result of evaporation. This ultimately leads to more variable $\delta^{18}O$ values amongst trees of the same species during years with heavier rainfall.

The carbon isotope variability within species at the Pinery is quite small (<2 ‰). It may be related to microclimatic variations in sunlight or temperature or both, but the available data are not conclusive.

6.3 The isotopic composition of pollen compared to stem and leaf cellulose

The extent of similarity between the oxygen isotope composition of pollen and stem and leaf cellulose depends on plant type and associated sugar translocation pathways. In the grasses and cattails studied, the $\delta^{18}O$ value of cellulose in the coexisting stems and leaves is higher than that of coexisting pollen. This occurs because the translocation pathway used by sugars during stem and leaf production is not the same as the pathway travelled by the sugars used in pollen synthesis. The first pathway provides less opportunity than the second for isotopic exchange with lower ^{18}O soil-water following initial sugar formation in the leaf.

Overall, the oxygen isotope composition of stem and leaf cellulose in trees are closer to those of pollen than in the grasses or cattails. This is because sugars used to build stem and leaf cellulose and pollen follow a similar route and are much less affected by exchange with low-^{18}O soil water during translocation. However, the extent to which the oxygen isotope composition of pollen in trees differs from the oxygen isotope composition of its leaf and stem cellulose differs among tree species. The $\delta^{18}O$ values of stem and leaf cellulose of the deciduous *Q. rubra* is similar to pollen $\delta^{18}O$ values, because leaves are formed during the same growing season as pollen, and sugars have little interaction with ^{18}O-depleted soil water, as the case with grasses and cattails. The $\delta^{18}O$ values of stem and leaf cellulose in coniferous trees is different from pollen $\delta^{18}O$

values, because these evergreens retain their needles for up to three years. Single needles, therefore, do not reflect the oxygen isotope composition of any one particular growing season. Pollen from pine trees is available in ample supply each year, and prominent in the sediment record. Although the $\delta^{18}O$ value of its pollen grains does not represent the $\delta^{18}O$ value of stem and leaf cellulose, it can be considered a more accurate proxy of the $\delta^{18}O$ values of sugars in the year it was produced.

The carbon isotope composition of cellulose and pollen in ever-green species did not differ significantly; however, in the deciduous *Q. rubra*, the $\delta^{13}C$ values of cellulose in leaves and stems were lower than pollen. This is likely a result of a higher amount of epicuticular waxes, which contain more ^{18}O-depleted lipids.

6.4 Future directions

The results of this thesis suggest several areas for future research:

• The data reported here suggest that the oxygen isotope composition of pollen depends largely on the extent to which sugars utilized in pollen formation retain $\delta^{18}O$ values acquired during their formation in the leaf. Growing-chamber studies are needed in which source water oxygen isotope composition is controlled, along with temperature, relative humidity, daylight hours and luminosity. Stem and leaf tissues (including isolated cellulose), pollen, stem and leaf water, and sugars can then be sampled at various stages of plant development. A range of C_3 and C_4 plant species that are amenable to growing chamber experiments should be examined. Similar experiments for tree species will be more challenging, but should be possible through a combination of controlled test plots and greenhouse facilities that can accommodate taller vegetation.

• The growing-chamber experiments described above should also be used to explore causes of intra-species variation in pollen $\delta^{13}C$ values, especially the putative roles of sunlight amount and temperature.

• The relationship between pollen carbon isotope composition and relative humidity reported by Loader and Hemming (2004) should be explored further. Species that grow over a wide range of relative humidity should be targeted, with an effort made to include deciduous and coniferous trees, a range of C_3 and C_4 grasses, and rooted marsh plants such as cattails and rooted macrophytes.

• The relationship between the oxygen isotope composition of pollen in trees and water availability should be explored more fully. At Pinery Provincial Park, ground-water, soil-water, and plant-water oxygen isotope compositions associated with *P. resinosa*, *P. strobus* and *Q. rubra* should be measured at critical periods during sugar formation in the leaves and subsequent pollen formation to track how different rooting depths may have affected pollen versus stem and leaf $\delta^{18}O$ values. Detailed soil-water oxygen and hydrogen isotope profiles, and their relationship to relative humidity, temperature and rain events, should be measured at the specific sites of the trees being studied, and the relationship between soil-water and root-water (and stem-water) oxygen isotopic compositions established for roots at various depths in the soil- and groundwater continuum.

• The nitrogen cycle in Pinery Provincial Park should be investigated more fully to determine the sources, types, and $\delta^{15}N$ values of bioavailable nitrogen in its forested and open dune areas. This is necessary to better understand the factors controlling pollen $\delta^{15}N$ values in the deciduous and coniferous trees, C_3 and C_4 grasses, and marsh plants such as cattails. This requires determination of the types, amounts, and isotopic compositions of bioavailable nitrogen compounds throughout the soil profiles, both in the forested areas and the open dunes (especially adjacent to Lake Huron), as well as systematic examination of the nitrogen isotope compositions of various plant parts formed in response from those sources. Such studies will be an effective complement to the isotopic examination of soil-water profiles described earlier.

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Appendices

Appendix One lists the oxygen, carbon, and nitrogen isotope compositions of pollen for tree, grass, and marsh species at Pinery Provincial Park, Ontario, from 2009 to 2012.

Appendix Two lists the oxygen, carbon, and nitrogen isotope compositions of pollen for tree species across North America for 2011 and 2012.

Appendix Three lists the oxygen and carbon isotope compositions of stem and leaf cellulose for tree, grass and marsh species collected during pollen fly at the Pinery in 2011.

Appendix Four lists the carbon and nitrogen percentages and ratios for all pollen samples.

Appendix Five lists temperature, relative humidity, and precipitation amount data collected at Pinery Provincial Park, Ontario and Goderich, Ontario from 2008 to 2012. The Pinery data were collected using three El-Win-USB dataloggers placed at (1) 43˚15’52.2684”N, 81˚48’57.0636”W; (2) 43˚16’54.5088”N, 81˚48’28.3356”W; and (3) 43˚16’29.568”N, 81˚49’17.2524”W. The Goderich data were obtained from The Weather Network (http://www.theweathernetwork.com, 2015).

Appendix Six lists the monthly oxygen isotope composition of precipitation collected from an automatic sampler in the Pinery from June, 2008 to November, 2012.

Appendix 1 Oxygen, carbon, and nitrogen isotope compositions of pollen for tree, grass and marsh species at Pinery Provincial Park, Ontario, from 2009 to 2012.

Sample Name	Year	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{13}C ‰ (VPDB) Pollen	δ^{15}N ‰ (AIR) Pollen	Δ^{18}O ‰ (VSMOW) Precipitation [1]
P. resinosa						
09RP-01	2009	43˚14’18.0528”N 81˚52’52.4568”W	+29.8	–26.6	–5.1	+14.6
09RP-02	2009	43˚14’23.5428”N 81˚50’46.4172”W	+29.2	–26.1	–4.4	+14.0
09RP-03	2009	43˚14’26.2716”N 81˚50’42.0324”W	+30.5	–26.4	–4.8	+15.3
09RP-04	2009	43˚17’08.5308”N 81˚48’05.7492”W	+28.7	–27.2	–4.0	+13.5
09RP-05	2009	43˚14’02.4360”N 81˚51’58.8168”W	+27.7	–26.5	–4.5	+12.5
09RP-06	2009	43˚16’48.2304”N 81˚48’37.6524”W	+29.3	–26.7	–4.1	+14.1
10RP-01	2010	43˚14’06.2088”N 81˚52’06.5712”W	+27.6	–27.7	–5.2	+11.5
10RP-02	2010	43˚17’02.9076”N 81˚48’15.4836”W	+27.3	–28.3	–5.4	+11.2
10RP-03	2010	43˚16’39.5976”N 81˚48’51.9840”W	+25.7	–27.7	–4.6	+9.6
10RP-04	2010	43˚17’05.0460”N 81˚48’10.9260”W	+26.7	–28.7	–5.5	+10.6
10RP-05	2010	43˚14’18.0528”N 81˚52’52.4568”W	+27.9	–27.7	–4.6	+11.9
10RP-06	2010	43˚16’53.0400”N 81˚48’32.2452”W	+28.0	–26.3	–4.4	+9.1
11RP-01	2011	43˚17’02.9076”N 81˚48’15.4836”W	+25.2	–28.6	–5.6	+8.3
11RP-02	2011	43˚16’53.0400”N 81˚48’32.2452”W	+24.4	–27.6	–4.5	+9.4
11RP-03	2011	43˚14’59.1612”N 81˚50’53.4156”W	+25.5	–27.2	–2.2	+9.2
11RP-04	2011	43˚14’06.2088”N 81˚52’06.5712”W	+25.3	–27.4	–4.4	+9.4
11RP-05	2011	43˚14’18.0528”N 81˚52’52.4568”W	+25.3	–28.2	–5.1	+9.0

[1] $\Delta^{18}O_{precipitation}$ is the difference between calculated δ^{18}O values of water (measured $\delta^{18}O_{pollen}$ values – 27‰) and $\delta^{18}O_{precipitation}$.

Appendix 1 Continued.

Sample Name	Year	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{13}C ‰ (VPDB) Pollen	δ^{15}N ‰ (AIR) Pollen	Δ^{18}O ‰ (VSMOW) Precipitation
P. resinosa						
11RP-06	2011	43˚17’11.0904”N 81˚48’03.6252”W	+25.5	–27.9	–3.8	+14.0
11RP-07	2011	43˚17’08.5308”N 81˚48’05.7492”W	+25.1	–28.3	–2.5	+15.3
11RP-08	2011	43˚14’26.2716”N 81˚50’42.0324”W	+25.7	–28.2	–5.2	+9.6
12RP-01	2012	43˚17’11.0904”N 81˚48’03.6252”W	+26.3	–27.9	–3.7	+11.1
12RP-02	2012	43˚16’53.0400”N 81˚48’32.2452”W	+26.3	–27.4	–4.5	+10.7
12RP-03	2012	43˚17’02.9076”N 81˚48’15.4836”W	+26.8	–27.6	–5.8	+11.2
12RP-04	2012	43˚16’21.2340”N 81˚49’24.8124”W	+26.2	–28.3	–6.5	+10.6
12RP-05	2012	43˚14’26.2716”N 81˚50’42.0324”W	+26.5	–27.8	–5.4	+10.9
12RP-06	2012	43˚17’08.5308”N 81˚48’05.7492”W	+25.7	–28.1	–2.6	+10.1
12RP-07	2012	43˚14’04.9992”N 81˚52’06.6072”W	+27.6	–28.3	–5.3	+12.0
12RP-08	2012	43˚14’18.0528”N 81˚52’52.4568”W	+26.7	–27.9	–5.4	+9.8
12RP-09	2012	43˚16’23.9916”N 81˚48’10.5984”W	+25.4	–28.8	–5.2	+11.2
12RP-10	2012	43˚14’59.1612”N 81˚50’53.4156”W	+26.8	–27.3	–2.6	+9.6
P. strobus						
09WP-01	2009	43˚15’52.2684”N 81˚48’57.0636”W	+22.6	–29.5	–3.3	+7.4
10WP-01	2010	43˚15’52.2684”N 81˚48’57.0636”W	+27.7	–26.8	–3.6	+11.1
10WP-02	2010	43˚16’20.1108”N 81˚49’26.5116”W	+27.2	–28.0	–4.6	+10.3
10WP-03	2010	43˚16’39.5688”N 81˚48’51.1344”W	+26.4	–28.4	–5.5	+10.6
10WP-04	2010	43˚17’10.3020”N 81˚48’05.0544”W	+26.7	–30.0	–4.4	+12.0
11WP-01	2011	43˚16’20.1108”N 81˚49’26.5116”W	+28.1	–26.3	–4.5	+10.9

Appendix 1 Continued.

Sample Name	Year	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{13}C ‰ (VPDB) Pollen	δ^{15}N ‰ (AIR) Pollen	Δ^{18}O ‰ (VSMOW) Precipitation
P. strobus						
11WP-02	2011	43˚16’30.0360”N 81˚49’04.8468”W	+27.0	–27.9	–3.9	+11.4
11WP-03	2011	43˚17’12.2424”N 81˚48’02.9700”W	+27.5	–29.6	–4.4	+11.1
11WP-04	2011	43˚16’27.0552”N 81˚49’13.4184”W	+27.9	–26.0	–4.2	+11.1
11WP-05	2011	43˚17’10.3020”N 81˚48’05.0544”W	+27.2	–27.1	–6.1	+11.8
11WP-06	2011	43˚15’52.2684”N 81˚48’57.0636”W	+27.9	–26.5	–3.8	+9.4
12WP-01	2012	43˚16’47.8092”N 81˚48’41.2452”W	+25.0	–26.6	-	+11.5
12WP-02	2012	43˚17’10.3020”N 81˚48’05.0544”W	+27.1	–26.9	–7.2	+11.5
12WP-03	2012	43˚15’52.2684”N 81˚48’57.0636”W	+27.6	–26.5	-	+11.6
12WP-04	2012	43˚17’12.2424”N 81˚48’02.9700”W	+27.1	–26.2	–7.0	+11.1
12WP-05	2012	43˚16’20.1108”N 81˚49’26.5116”W	+27.2	–25.9	–5.6	+11.8
Q. rubra						
09RO-01	2009	43˚16’23.0052”N 81˚48’08.2224”W	+30.6	–27.3	–3.2	+15.4
10RO-01	2010	43˚17’08.5308”N 81˚48’05.7888”W	+25.2	–27.1	–4.1	+9.1
10RO-02	2010	43˚17’07.8000”N 81˚48’08.8020”W	+24.0	–24.9	–3.4	+7.9
10RO-03	2010	43˚17’08.0520”N 81˚48’08.3772”W	+25.3	–24.9	–3.3	+9.2
11RO-01	2011	43˚17’08.0520”N 81˚48’08.3772”W	+26.5	–26.4	–4.3	+10.4
11RO-02	2011	43˚16’38.4744”N 81˚48’54.1836”W	+26.3	–26.6	–4.2	+10.2
11RO-03	2011	43˚16’26.4936”N 81˚49’13.1880”W	+26.1	–26.6	–4.7	+10.0
11RO-04	2011	43˚16’21.3204”N 81˚49’23.2320”W	+26.4	–26.1	–3.5	+10.3
11RO-05	2011	43˚16’24.4704”N 81˚48’11.3112”W	+26.1	–26.8	–1.7	+10.0

Appendix 1 Continued.

Sample Name	Year	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{13}C ‰ (VPDB) Pollen	δ^{15}N ‰ (AIR) Pollen	Δ^{18}O ‰ (VSMOW) Precipitation
Q. rubra						
12RO-01	2012	43˚16’21.3204”N 81˚49’23.2320”W	+25.5	–25.4	–3.1	+9.9
12RO-02	2012	43˚17’07.8000”N 81˚48’08.8020”W	+24.6	–25.0	–2.7	+9.0
12RO-03	2012	43˚17’01.1076”N 81˚48’17.9172”W	+25.5	–26.7	–5.6	+9.9
12RO-04	2012	43˚16’53.2344”N 81˚48’30.0636”W	+26.2	–27.3	–3.5	+10.6
12RO-05	2012	43˚16’47.5284”N 81˚48’39.9312”W	+25.1	–26.4	–4.2	+9.5
12RO-06	2012	43˚16’30.6552”N 81˚49’02.2584”W	+25.3	–26.6	–2.5	+9.7
12RO-07	2012	43˚16’26.4936”N 81˚49’13.1880”W	+27.5	–26.7	–3.7	+11.9
12RO-08	2012	43˚16’21.3204”N 81˚49’23.2320”W	+26.0	–27.0	–3.1	+10.4
A. breviligulata						
09AM-01	2009	43˚16’08.2416”N 81˚49’57.5004”W	+24.9	–25.4	+0.9	+5.2
10AM-01	2010	43˚16’08.2416”N 81˚49’57.5004”W	+25.4	–25.3	+1.5	+4.4
11AM-01	2011	43˚16’08.2416”N 81˚49’57.5004”W	+22.4	–26.5	+2.3	+2.0
12AM-01	2012	43˚16’08.2416”N 81˚49’57.5004”W	+21.9	–25.8	+3.5	–1.0
A. gerardii						
10BB-01	2010	43˚16’06.0348”N 81˚49’47.3412”W	+26.3	–11.3	–1.8	+5.3
11BB-01	2011	43˚16’06.0348”N 81˚49’47.3412”W	+25.4	–12.9	–1.8	+5.0
12BB-01	2012	43˚16’06.0348”N 81˚49’47.3412”W	+21.6	–12.4	–3.5	–1.3
T. latifolia						
10CA-01	2010	43˚24’57.2004”N 81˚40’32.1528”W	+21.1	–28.8	+11.4	–0.3
10CA-02	2010	42˚59’53.4948”N 81˚16’31.0512”W	+21.8	–30.1	+9.2	+0.4
11CA-01	2011	43˚24’57.2004”N 81˚40’32.1528”W	+20.7	–29.1	+9.8	+1.0

Appendix 1 Continued.

Sample Name	Year	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{13}C ‰ (VPDB) Pollen	δ^{15}N ‰ (AIR) Pollen	Δ^{18}O ‰ (VSMOW) Precipitation
T. latifolia						
11CA-02	2011	42˚59’53.4948”N 81˚16’31.0512”W	+23.9	–29.5	+8.4	+4.2
12CA-02	2012	42˚59’53.4948”N 81˚16’31.0512”W	+26.2	–28.4	+6.3	+3.3
12CA -03	2012	42˚59’53.4948”N 81˚16’31.0512”W	+25.2	–28.9	+7.0	+2.3
12CA -04	2012	42˚59’53.4948”N 81˚16’31.0512”W	+26.6	–27.5	+6.6	+3.7
12CA -05	2012	42˚59’53.4948”N 81˚16’31.0512”W	+25.9	–28.1	+7.3	+3.0
12CA -06	2012	42˚59’53.4948”N 81˚16’31.0512”W	+24.9	–28.2	+6.1	+2.0
12CA -07	2012	42˚59’53.4948”N 81˚16’31.0512”W	+24.4	–27.9	+5.9	+1.5
12CA -08	2012	42˚59’53.4948”N 81˚16’31.0512”W	+25.1	–28.0	+6.6	+2.2
12CA -09	2012	42˚59’53.4948”N 81˚16’31.0512”W	+25.4	–28.0	+7.3	+2.5
12CA -10	2012	42˚59’53.4948”N 81˚16’31.0512”W	+26.0	–28.5	+6.2	+3.1
12CA -11	2012	43˚24’57.2004”N 81˚40’32.1528”W	+24.1	–29.3	+10.5	+1.2
12CA-13	2012	43˚24’57.2004”N 81˚40’32.1528”W	+23.4	–30.3	+11.1	+0.5

Appendix 2 Oxygen, carbon, and nitrogen isotope compositions of pollen for tree species across North America for 2011 and 2012.

Species	Year	Sample Name	Location	δ^{18}O ‰ (VSMOW) Pollen	δ^{18}O ‰ (VSMOW) Precipitation[1]	δ^{13}C ‰ (VPDB) Pollen	Relative Humidity %[2]	δ^{15}N ‰ (AIR) Pollen
Q. stellata	2011	11-USA-TX-01	Texas	+32.0	–4.8	–28.5	72	+2.7
Q. comptoniae	2011	11-USA-TX-02	Texas	+31.0	–4.8	–27.6	72	+5.3
Q. shumardii	2011	11-USA-TX-03	Texas	+32.2	–4.8	–26.3	72	+0.9
Q. virginiana	2011	11-USA-TX-04	Texas	+32.3	–4.8	–29.1	72	+0.5
Q. macrocarpa	2011	11-USA-ND-01	North Dakota	+21.6	–16.5	–27.1	62	+4.8
Q. stellata	2011	11-USA-AR-01	Arkansas	+28.7	–6.5	–26.7	67	+0.5
Q. shumardii	2011	11-USA-AR-02	Arkansas	+27.9	–6.3	–26.6	67	–0.2
Q. virginiana	2011	11-USA-FL-01	Florida	+27.8	–5.1	–28.1	68	+0.8
Q. virginiana	2011	11-USA-FL-02	Florida	+26.2	–5.1	–29.0	71	–0.1
A. saccharium	2011	11-USA-SD-01	South Dakota	+23.1	–12.2	–22.2	69	+5.8
A. saccharium	2011	11-USA-SD-02	South Dakota	+23.8	–12.2	–22.1	69	+5.7
A. saccharium	2011	11-USA-SD-03	South Dakota	+24.4	–12.2	–24.9	69	+3.5
Q. marilandica	2012	12-USA-TX-01	Texas	+27.0	–4.8	–24.5	72	–0.2
Q. shumardii	2012	12-USA-TX-02	Texas	+27.0	–4.8	–25.8	72	+0.4
Q. stellata	2012	12-USA-TX-03	Texas	+27.2	–4.8	–25.8	72	+3.2
Q. macrocarpus	2012	12-USA-TX-04	Texas	+28.3	–4.8	–25.0	72	+3.1
Q. nigra	2012	12-USA-TX-05	Texas	+26.8	–4.8	–27.7	72	+2.2
Q. virginiana	2012	12-USA-FL-01	Florida	+27.5	–5.1	–27.8	68	+0.6
Q. virginiana	2012	12-USA-FL-02	Florida	+28.4	–5.1	–29.4	71	–1.2
A. macrophyllum	2012	12-USA-WA-01	Washington	+23.0	–10.8	–24.8	78	–0.3
Q. stellata	2012	12-USA-AR-01	Arkansas	+29.9	–6.5	–27.8	67	–0.5
Q. shumardii	2012	12-USA-AR-02	Arkansas	+30.2	–6.3	–27.8	67	–0.4

[1] Precipitation δ^{18}O values were calculated for October and November of the previous growing season, using the algorithm of Bowen and Revenaugh (2003).
[2] Relative humidity values are the average for October/November of the previous growing season (obtained from www.myforecast.com).

Appendix 3 Oxygen and carbon isotope compositions of stem and leaf cellulose for tree, grass and marsh species collected during pollen fly at the Pinery in 2011.

Sample Name	Location	Plant Part	δ^{18}O ‰ (VSMOW) Cellulose	δ^{13}C ‰ (VPDB) Cellulose
P. resinosa				
RP-N-AM01	43˚17’02.9076”N 81˚48’15.4836”W	Needle	+28.5	–27.4
RP-N-AM02	43˚17’02.9076”N 81˚48’15.4836”W	Needle	+28.6	–27.9
RP-St-AM01	43˚17’02.9076”N 81˚48’15.4836”W	Stem	+28.5	–27.2
RP-St-AM02	43˚17’02.9076”N 81˚48’15.4836”W	Stem	+29.3	–27.3
P. strobus				
WP-N-AM01	43˚15’52.2684”N 81˚48’57.0636”W	Needle	+28.2	–27.6
WP-N-AM02	43˚15’52.2684”N 81˚48’57.0636”W	Needle	+29.2	–27.5
WP-St-AM01	43˚15’52.2684”N 81˚48’57.0636”W	Stem	+27.7	–27.7
WP-St-AM02	43˚15’52.2684”N 81˚48’57.0636”W	Stem	+28.4	–27.3
Q. rubra				
RO-L-AM01	43˚17’07.8000”N 81˚48’08.8020”W	Leaf	+27.9	–27.6
RO-L-AM02	43˚17’07.8000”N 81˚48’08.8020”W	Leaf	+28.4	–29.2
RO-St-AM01	43˚17’07.8000”N 81˚48’08.8020”W	Stem	+25.7	–26.7
RO-St-AM02	43˚17’07.8000”N 81˚48’08.8020”W	Stem	+25.3	–29.0
A. breviligulata				
AM-L-AM01	43˚16’08.2416”N 81˚49’57.5004”W	Leaf	+27.6	–25.8
AM-L-AM02	43˚16’08.2416”N 81˚49’57.5004”W	Leaf	+27.9	–25.4
AM-St-AM01	43˚16’08.2416”N 81˚49’57.5004”W	Stem	+27.0	–25.9
AM-St-AM02	43˚16’08.2416”N 81˚49’57.5004”W	Stem	+26.9	–26.2

Appendix 3 Continued

Sample Name	Location	Plant Part	δ^{18}O ‰ (VSMOW) Cellulose	δ^{13}C ‰ (VPDB) Cellulose
A. gerardii				
BB-L-AM01	43˚16’06.0348”N 81˚49’47.3412”W	Leaf	+30.8	–11.5
BB-L-AM02	43˚16’06.0348”N 81˚49’47.3412”W	Leaf	+30.6	–12.0
BB-St-AM01	43˚16’06.0348”N 81˚49’47.3412”W	Stem	+31.0	–12.2
BB-St-AM02	43˚16’06.0348”N 81˚49’47.3412”W	Stem	+29.8	–12.8
T. latifolia				
CA-L01	43˚24’57.2004”N 81˚40’32.1528”W	Leaf	+27.6	–27.3
CA-St01	43˚24’57.2004”N 81˚40’32.1528”W	Stem	+28.6	–27.0

Appendix 4 Carbon and nitrogen weight % and atom % ratios for all pollen samples.

Sample Name	Carbon wt. %	Nitrogen wt. %	C/N (at. %)
Pollen			
09AM-01	44.0	2.9	18.0
09RO-01	52.0	5.5	11.0
09WP-01	54.9	2.4	26.7
09RP-01	49.7	2.3	25.0
09RP-02	49.0	2.3	25.0
09RP-03	51.1	2.1	28.6
09RP-04	51.4	2.1	28.7
09RP-05	51.4	2.0	29.4
09RP-06	51.4	2.1	28.3
10RP-01	49.6	2.1	27.8
10RP-02	48.7	2.0	28.5
10RP-03	44.3	2.4	21.4
10RP-04	49.0	2.0	28.5
10RP-05	48.4	2.1	26.7
10RP-06	47.9	1.9	29.8
10WP-01	49.1	2.1	27.6
10WP-02	49.2	2.0	28.4
10WP-03	49.2	1.9	29.5
10WP-04	48.1	2.0	28.0
10RO-01	49.8	6.1	9.6
10RO-02	47.3	6.3	8.8
10RO-03	49.8	6.1	9.5
10CA-01	45.7	3.2	16.8
10CA-02	47.4	3.3	16.9
10BB-01	49.5	3.6	16.2
10AM-01	41.7	3.2	15.3
11RO-01	49.6	6.0	9.6
11RO-02	47.5	6.4	8.6
11R0-03	50.4	6.1	9.7
11RO-04	49.3	6.2	9.3
11RO-05	48.1	6.6	8.6
11RP-01	43.3	1.7	28.9
11RP-02	47.1	1.7	33.1
11RP-03	42.5	1.3	37.8
11RP-04	43.8	2.2	23.3
11RP-05	39.5	1.7	27.9
11RP-06	45.5	1.9	28.2
11RP-07	47.3	2.2	25.0

Appendix 4 Continued.

Sample Name	Carbon wt. %	Nitrogen wt. %	C/N (at. %)
Pollen			
11RP-08	36.0	1.5	27.2
11WP-01	44.8	2.0	25.8
11WP-02	39.1	2.1	21.8
11WP-03	46.1	2.1	26.0
11WP-04	48.8	2.2	25.3
11WP-05	29.5	1.5	23.5
11WP-06	48.6	2.1	26.6
11CA-01	52.0	2.9	20.6
11CA-02	45.4	2.8	18.6
11BB-01	52.3	3.6	16.9
11AM-01	42.6	4.1	12.1
11-USA-TX-01	51.4	5.1	11.7
11-USA-TX-02	50.1	3.7	15.7
11-USA-TX-03	49.8	6.4	9.0
11-USA-TX-04	49.0	6.2	9.2
11-USA-ND-01	54.3	5.3	11.9
11-USA-AR-01	55.0	5.8	11.2
11-USA-AR-02	46.1	5.3	10.1
11-USA-FL-01	50.0	5.8	10.0
11-USA-FL-02	51.8	4.2	14.4
11-USA-SD-01	47.2	6.8	8.1
11-USA-SD-02	51.3	6.7	8.9
11-USA-SD-03	50.9	6.6	9.0
12RP-01	49.0	2.7	21.0
12RP-02	44.1	2.4	21.7
12RP-03	51.4	2.6	23.5
12RP-04	50.8	2.7	22.3
12RP-05	49.7	3.1	18.6
12RP-06	48.1	2.0	28.2
12RP-07	49.6	2.1	27.0
12RP-08	49.1	2.7	21.3
12RP-09	47.4	2.8	19.9
12RP-10	39.1	2.5	18.2
WP12-01	48.6	2.7	20.8
WP12-02	40.9	2.3	21.1
WP12-03	49.1		
WP12-04	50.1	2.7	21.7
WP12-05	45.8	2.3	23.4

Appendix 4 Continued.

Sample Name	Carbon wt. %	Nitrogen wt. %	C/N (at. %)
Pollen			
RO12-01	50.7	5.8	10.2
RO12-02	49.5	5.2	11.1
RO12-03	48.3	5.9	9.6
RO12-04	51.3	6.2	9.6
RO12-05	42.2	6.3	7.8
RO12-06	50.9	5.7	10.4
RO12-07	42.0	5.8	8.4
RO12-08	44.4	6.2	8.4
CA12-05	48.7	3.0	19.2
CA12-06	47.1	3.3	16.4
CA12-08	42.7	3.2	15.5
CA12-09	49.2	3.1	18.6
CA12-10	47.3	3.1	17.6
CA12-11	48.5	3.2	17.8
CA12-13	51.8	3.1	19.7
CA12-07	42.8	3.4	14.6
12BB-01	47.6	3.6	15.3
12AM-01	41.9	3.6	13.6
12-USA-TX-01	51.9	5.0	12.0
12-USA-TX-02	46.6	6.1	8.8
12-USA-TX-03	47.1	5.1	10.7
12-USA-TX-04	50.1	5.8	10.1
12-USA-TX-05	48.1	5.7	9.9
12-USA-FL-01	45.6	4.9	10.8
12-USA-FL-02	48.6	5.5	10.4
12-USA-WA-01	45.7	6.2	8.6
12-USA-AR-01	46.7	5.9	9.2
12-USA-AR-02	48.5	5.9	9.6

Appendix 5 Environmental data recorded for southwestern Ontario (Pinery, Goderich).

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
October 1, 2008	Goderich	+12.9	+8.1	87	15.2
October 2, 2008	Goderich	+12.9	+6.9	74	19.3
October 3, 2008	Goderich	+11.5	+4.3	60	5.1
October 4, 2008	Goderich	+12.8	+7.9	60	1.1
October 5, 2008	Goderich	+14.4	+6.2	60	0.0
October 6, 2008	Goderich	+13.9	+4.8	40	0.0
October 7, 2008	Goderich	+15.3	+1.9	43	0.0
October 8, 2008	Goderich	+16.0	+10.3	95	7.9
October 9, 2008	Goderich	+18.0	+12.6	68	0.0
October 10, 2008	Goderich	+16.4	+5.6	64	0.0
October 11, 2008	Goderich	+21.4	+7.2	55	0.0
October 12, 2008	Goderich	+24.6	+12.3	41	0.2
October 13, 2008	Goderich	+24.2	+10.5	39	0.0
October 14, 2008	Goderich	+20.3	+5.0	65	0.4
October 15, 2008	Goderich	+15.3	+7.3	84	8.3
October 16, 2008	Goderich	+12.1	+6.8	65	0.9
October 17, 2008	Goderich	+12.3	+2.4	55	0.0
October 18, 2008	Goderich	+12.0	+1.5	45	0.0
October 19, 2008	Goderich	+12.8	+2.9	55	0.0
October 20, 2008	Goderich	+12.7	+6.8	75	12.0
October 21, 2008	Goderich	+8.9	+1.0	73	3.2
October 22, 2008	Goderich	+8.6	–0.6	50	0.0
October 23, 2008	Goderich	+12.0	–3.5	58	0.0
October 24, 2008	Goderich	+10.6	+3.6	70	4.6

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
October 25, 2008	Goderich	+10.7	+9.0	66	3.6
October 26, 2008	Goderich	+11.5	+5.9	86	6.3
October 27, 2008	Goderich	+7.7	+3.7	89	14.3
October 28, 2008	Goderich	+4.4	+1.5	91	6.1
October 29, 2008	Goderich	+4.6	+1.6	60	0.4
October 30, 2008	Goderich	+9.0	–0.5	64	0.0
October 31, 2008	Goderich	+15.6	+5.1	60	0.2
November 1, 2008	Goderich	+9.2	–0.6	60	0.0
November 2, 2008	Goderich	+9.0	–3.1	50	6.1
November 3, 2008	Goderich	+14.6	+7.0	84	20.6
November 4, 2008	Goderich	+19.0	+10.3	49	0.0
November 5, 2008	Goderich	+19.7	+9.0	42	0.0
November 6, 2008	Goderich	+20.4	+7.2	46	0.0
November 7, 2008	Goderich	+17.1	+8.9	69	14.1
November 8, 2008	Goderich	+8.9	+4.1	79	2.2
November 9, 2008	Goderich	+5.1	+2.0	76	0.6
November 10, 2008	Goderich	+4.4	+0.6	69	1.2
November 11, 2008	Goderich	+4.4	+2.0	68	0.4
November 12, 2008	Goderich	+7.5	+1.5	68	0.2
November 13, 2008	Goderich	+10.9	+5.9	94	13.9
November 14, 2008	Goderich	+11.9	+6.4	86	9.0
November 15, 2008	Goderich	+6.4	+1.6	91	25.7
November 16, 2008	Goderich	+3.1	–0.8	73	5.5
November 17, 2008	Goderich	+1.5	–5.0	76	4.2
November 18, 2008	Goderich	–3.2	–6.6	86	5.2

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
November 19, 2008	Goderich	+1.5	–5.3	84	5.2
November 20, 2008	Goderich	+2.4	–4.7	79	7.0
November 21, 2008	Goderich	–3.0	–8.7	95	4.4
November 22, 2008	Goderich	–2.0	–7.2	91	3.6
November 23, 2008	Goderich	–0.4	–6.3	78	21.4
November 24, 2008	Goderich	+3.3	–1.1	76	7.8
November 25, 2008	Goderich	+3.3	+0.7	93	5.1
November 26, 2008	Goderich	+2.6	+1.1	79	2.2
November 27, 2008	Goderich	+2.7	+0.7	72	0.7
November 28, 2008	Goderich	+3.0	+0.0	96	2.7
November 29, 2008	Goderich	+2.9	–3.9	67	0.0
November 30, 2008	Goderich	+1.9	–2.8	73	11.4
March 1, 2009	Goderich	–7.8	–14.0	78	0.0
March 2, 2009	Goderich	–12.6	–17.5	72	0.0
March 3, 2009	Goderich	–7.8	–15.8	72	0.0
March 4, 2009	Goderich	–1.8	–15.5	66	0.0
March 5, 2009	Goderich	+11.2	–5.3	56	0.0
March 6, 2009	Goderich	+12.6	–1.8	80	0.0
March 7, 2009	Goderich	+3.4	–2.2	95	15.4
March 8, 2009	Goderich	+6.6	–0.2	94	12.9
March 9, 2009	Goderich	+4.0	–3.0	93	3.4
March 10, 2009	Goderich	+8.6	–0.6	91	10.6
March 11, 2009	Goderich	+10.7	–6.3	77	3.3
March 12, 2009	Goderich	–6.2	–9.4	71	0.0
March 13, 2009	Goderich	–1.3	–9.4	72	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 14, 2009	Goderich	+5.6	–3.6	69	0.0
March 15, 2009	Goderich	+8.2	–2.6	62	0.0
March 16, 2009	Goderich	+10.4	–1.7	70	0.0
March 17, 2009	Goderich	+15.6	–1.2	61	0.0
March 18, 2009	Pinery	+12.1	–2.0	46	0.0
March 19, 2009	Pinery	+1.0	–5.9	66	0.0
March 20, 2009	Pinery	+0.0	–7.8	60	0.0
March 21, 2009	Pinery	+3.6	–3.9	66	0.0
March 22, 2009	Pinery	+4.7	–5.9	71	0.0
March 23, 2009	Pinery	+2.8	–7.4	72	0.0
March 24, 2009	Pinery	+8.0	–2.8	39	0.0
March 25, 2009	Pinery	+8.9	+3.2	67	4.6
March 26, 2009	Pinery	+6.5	+0.2	81	0.0
March 27, 2009	Pinery	+12.9	–1.5	69	0.0
March 28, 2009	Pinery	+11.2	–1.3	80	0.0
March 29, 2009	Pinery	+9.8	–0.9	78	17.1
March 30, 2009	Pinery	+0.9	–3.6	81	0.0
March 31, 2009	Pinery	+10.7	–3.1	73	0.0
April 1, 2009	Pinery	+9.8	+0.6	63	4.5
April 2, 2009	Pinery	+16.4	+1.7	52	1.7
April 3, 2009	Pinery	+11.9	+1.0	87	21.5
April 4, 2009	Pinery	+4.4	+0.6	76	0.0
April 5, 2009	Pinery	+4.5	–1.0	81	0.7
April 6, 2009	Pinery	+1.2	–3.4	92	7.2
April 7, 2009	Pinery	–0.6	–4.0	79	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
April 8, 2009	Pinery	+5.9	–1.7	56	0.0
April 9, 2009	Pinery	+9.6	–2.0	55	0.0
April 10, 2009	Pinery	+8.8	–1.9	70	0.0
April 11, 2009	Pinery	+6.8	–3.2	64	0.0
April 12, 2009	Pinery	+2.9	–4.6	64	0.0
April 13, 2009	Pinery	+10.6	–4.6	49	0.0
April 14, 2009	Pinery	+10.2	+4.4	33	0.0
April 15, 2009	Pinery	+17.1	+4.4	43	0.0
April 16, 2009	Pinery	+9.5	–1.1	69	0.0
April 17, 2009	Pinery	+16.3	–3.4	52	0.0
April 18, 2009	Pinery	+20.0	+8.4	43	0.0
April 19, 2009	Pinery	+14.8	+6.3	48	0.0
April 20, 2009	Pinery	+9.0	+4.5	80	7.3
April 21, 2009	Pinery	+10.2	+2.8	80	1.0
April 22, 2009	Pinery	+5.3	+1.8	80	0.0
April 23, 2009	Pinery	+9.6	+1.7	67	0.0
April 24, 2009	Pinery	+22.1	+7.6	53	0.0
April 25, 2009	Pinery	+24.6	+6.2	62	16.8
April 26, 2009	Pinery	+17.1	+6.5	91	8.0
April 27, 2009	Pinery	+24.9	+11.9	65	1.0
April 28, 2009	Pinery	+13.0	+2.2	89	10.2
April 29, 2009	Pinery	+15.5	+1.7	57	0.0
April 30, 2009	Pinery	+17.8	+8.5	76	13.7
May 1, 2009	Pinery	+12.0	+1.4	80	0.0
May 2, 2009	Pinery	+13.8	+0.7	65	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
May 3, 2009	Pinery	+17.7	+5.7	48	0.0
May 4, 2009	Pinery	+16.3	+5.0	53	0.0
May 5, 2009	Pinery	+17.5	+5.5	55	0.0
May 6, 2009	Pinery	+18.3	+7.9	59	0.0
May 7, 2009	Pinery	+15.6	+10.3	81	1.1
May 8, 2009	Pinery	+21.1	+8.7	76	0.0
May 9, 2009	Pinery	+14.5	+4.7	89	19.6
May 10, 2009	Pinery	+7.9	+1.7	75	0.0
May 11, 2009	Pinery	+9.9	+0.2	71	0.0
May 12, 2009	Pinery	+14.5	–1.0	61	0.0
May 13, 2009	Pinery	+20.9	+7.1	58	0.0
May 14, 2009	Pinery	+16.5	+7.9	68	10.2
May 15, 2009	Pinery	+19.1	+6.3	66	0.0
May 16, 2009	Pinery	+15.2	+4.3	82	12.9
May 17, 2009	Pinery	+9.8	+0.4	53	0.0
May 18, 2009	Pinery	+14.6	–1.5	54	0.4
May 19, 2009	Pinery	+20.5	+10.2	39	0.0
May 20, 2009	Pinery	+24.1	+15.5	39	0.0
May 21, 2009	Pinery	+25.3	+10.3	41	0.0
May 22, 2009	Pinery	+14.4	+6.4	81	0.0
May 23, 2009	Pinery	+20.8	+6.5	69	0.3
May 24, 2009	Pinery	+14.9	+4.7	78	0.0
May 25, 2009	Pinery	+20.0	+2.6	72	0.0
May 26, 2009	Pinery	+20.6	+9.4	52	0.0
May 27, 2009	Pinery	+22.1	+14.3	87	22.9

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
May 28, 2009	Pinery	+20.5	+9.6	95	2.8
May 29, 2009	Pinery	+19.6	+7.5	80	2.9
May 30, 2009	Pinery	+18.3	+5.4	72	6.2
May 31, 2009	Pinery	+11.1	+6.1	57	0.0
June 1, 2009	Pinery	+16.3	+6.7	81	1.9
June 2, 2009	Pinery	+13.0	+5.5	81	0.0
June 3, 2009	Pinery	+11.6	+2.9	80	0.0
June 4, 2009	Pinery	+12.4	+1.9	76	0.0
June 5, 2009	Pinery	+20.8	+5.5	63	0.0
June 6, 2009	Pinery	+11.7	+8.0	69	0.0
June 7, 2009	Pinery	+20.9	+9.4	73	0.0
June 8, 2009	Pinery	+17.8	+12.1	81	16.0
June 9, 2009	Pinery	+17.4	+10.2	84	0.0
June 10, 2009	Pinery	+16.2	+9.4	83	0.0
June 11, 2009	Pinery	+20.1	+11.2	90	0.0
June 12, 2009	Pinery	+18.1	+8.1	83	0.0
June 13, 2009	Pinery	+17.9	+8.7	78	0.0
June 14, 2009	Pinery	+21.5	+7.7	73	0.8
June 15, 2009	Pinery	+15.4	+9.0	82	0.0
June 16, 2009	Pinery	+25.2	+8.4	62	0.0
June 17, 2009	Pinery	+20.6	+15.6	84	16.0
June 18, 2009	Pinery	+17.4	+12.0	90	0.0
June 19, 2009	Pinery	+24.5	+12.4	82	0.0
June 20, 2009	Pinery	+18.7	+12.1	93	8.7
June 21, 2009	Pinery	+17.3	+11.8	93	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
June 22, 2009	Pinery	+19.2	+12.4	91	0.0
June 23, 2009	Pinery	+23.4	+14.9	84	0.0
June 24, 2009	Pinery	+29.0	+15.9	72	0.0
June 25, 2009	Pinery	+24.1	+18.1	79	8.7
June 26, 2009	Pinery	+19.2	+13.1	85	0.0
June 27, 2009	Pinery	+22.1	+11.7	77	0.3
June 28, 2009	Pinery	+24.4	+16.0	75	6.3
June 29, 2009	Pinery	+20.4	+15.1	74	9.4
June 30, 2009	Pinery	+16.3	+13.6	91	2.2
July 1, 2009	Pinery	+17.2	+12.8	90	0.0
July 2, 2009	Pinery	+17.7	+13.5	90	0.5
July 3, 2009	Pinery	+16.1	+12.2	90	0.4
July 4, 2009	Pinery	+19.8	+11.2	73	0.0
July 5, 2009	Pinery	+23.1	+10.5	73	1.3
July 6, 2009	Pinery	+21.2	+11.2	76	0.0
July 7, 2009	Pinery	+16.8	+11.2	76	0.3
July 8, 2009	Pinery	+16.0	+11.7	81	0.0
July 9, 2009	Pinery	+22.1	+11.7	81	0.3
July 10, 2009	Pinery	+26.4	+15.2	70	0.0
July 11, 2009	Pinery	+23.5	+12.2	77	22.5
July 12, 2009	Pinery	+21.4	+8.3	67	0.0
July 13, 2009	Pinery	+20.8	+8.4	64	0.0
July 14, 2009	Pinery	+21.1	+10.4	61	0.0
July 15, 2009	Pinery	+23.8	+13.3	73	2.2
July 16, 2009	Pinery	+23.6	+12.5	67	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
July 17, 2009	Pinery	+19.7	+12.2	77	0.0
July 18, 2009	Pinery	+17.9	+13.6	83	0.0
July 19, 2009	Pinery	+19.8	+12.7	76	0.0
July 20, 2009	Pinery	+21.9	+13.8	78	0.0
July 21, 2009	Pinery	+24.5	+13.3	82	1.7
July 22, 2009	Pinery	+25.6	+15.2	84	0.0
July 23, 2009	Pinery	+20.8	+17.3	91	8.1
July 24, 2009	Pinery	+23.1	+17.2	79	1.9
July 25, 2009	Pinery	+23.8	+16.7	87	8.4
July 26, 2009	Pinery	+21.3	+17.7	86	0.0
July 27, 2009	Pinery	+24.2	+17.5	76	0.0
July 28, 2009	Pinery	+25.6	+20.7	78	0.0
July 29, 2009	Pinery	+21.5	+13.3	83	0.0
July 30, 2009	Pinery	+23.0	+12.1	76	0.4
July 31, 2009	Pinery	+21.2	+14.8	81	0.0
August 1, 2009	Pinery	+25.2	+15.6	71	0.0
August 2, 2009	Pinery	+21.7	+18.4	66	0.0
August 3, 2009	Pinery	+24.2	+15.1	68	0.0
August 4, 2009	Pinery	+25.5	+12.2	75	0.0
August 5, 2009	Pinery	+21.4	+9.1	62	0.3
August 6, 2009	Pinery	+21.1	+10.8	73	0.3
August 7, 2009	Pinery	+19.2	+9.1	73	0.0
August 8, 2009	Pinery	+20.3	+12.7	84	8.3
August 9, 2009	Pinery	+29.0	+20.0	82	5.1
August 10, 2009	Pinery	+24.6	+17.4	84	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
August 11, 2009	Pinery	+22.8	+16.6	91	0.0
August 12, 2009	Pinery	+22.0	+15.4	85	0.0
August 13, 2009	Pinery	+23.2	+13.6	81	0.0
August 14, 2009	Pinery	+26.9	+14.8	79	0.0
August 15, 2009	Pinery	+28.1	+19.2	76	0.0
August 16, 2009	Pinery	+30.0	+19.4	75	0.0
August 17, 2009	Pinery	+26.9	+22.0	76	0.0
August 18, 2009	Pinery	+26.0	+16.1	77	5.7
October 17, 2009	Goderich	+8.7	–2.4	46	0.0
October 18, 2009	Goderich	+8.7	–4.2	46	0.0
October 19, 2009	Goderich	+13.8	+4.4	53	0.0
October 20, 2009	Goderich	+13.4	+8.6	73	0.0
October 21, 2009	Goderich	+16.6	+10.3	87	0.0
October 22, 2009	Goderich	+14.5	+4.0	91	0.0
October 23, 2009	Goderich	+14.7	+3.4	97	9.5
October 24, 2009	Goderich	+13.1	+6.8	80	0.0
October 25, 2009	Goderich	+12.0	+6.9	75	0.0
October 26, 2009	Goderich	+15.3	+9.3	53	0.0
October 27, 2009	Goderich	+11.5	+7.8	79	0.5
October 28, 2009	Goderich	+14.4	+4.5	70	0.9
October 29, 2009	Goderich	+12.8	+4.2	83	0.0
October 30, 2009	Goderich	+20.9	+10.0	82	24.3
October 31, 2009	Goderich	+19.3	+6.2	67	1.9
November 1, 2009	Goderich	+9.3	+4.3	70	0.0
November 2, 2009	Goderich	+9.6	+1.7	72	1.8

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
November 3, 2009	Goderich	+7.1	+3.0	64	0.0
November 4, 2009	Goderich	+7.2	+1.9	63	0.0
November 5, 2009	Goderich	+6.7	+2.5	52	5.4
November 6, 2009	Goderich	+7.9	–0.1	56	0.0
November 7, 2009	Goderich	+14.8	+7.8	55	0.0
November 8, 2009	Goderich	+18.7	+1.9	38	0.0
November 9, 2009	Goderich	+14.9	+4.7	79	0.0
November 10, 2009	Goderich	+10.8	+0.1	80	0.0
November 11, 2009	Goderich	+9.7	–1.7	50	0.0
November 12, 2009	Goderich	+9.8	–2.6	70	0.0
November 13, 2009	Goderich	+13.6	+3.0	35	0.0
November 14, 2009	Goderich	+17.6	+8.6	57	0.0
November 15, 2009	Goderich	+12.9	+3.9	81	0.0
November 16, 2009	Goderich	+7.3	–1.2	67	0.0
November 17, 2009	Goderich	+10.5	–1.0	43	0.0
November 18, 2009	Goderich	+13.1	+3.2	46	0.7
November 19, 2009	Goderich	+11.5	+6.9	92	0.9
November 20, 2009	Goderich	+8.6	+6.0	87	0.0
November 21, 2009	Goderich	+9.0	+7.3	89	0.0
November 22, 2009	Goderich	+9.4	+1.4	74	0.0
November 23, 2009	Goderich	+9.9	+2.8	72	0.0
November 24, 2009	Goderich	+9.2	+6.2	91	0.0
November 25, 2009	Goderich	+10.3	+7.4	90	4.2
November 26, 2009	Goderich	-	+5.6	74	0.5
November 27, 2009	Goderich	+6.7	+2.7	79	4.1

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
November 28, 2009	Goderich	+5.0	–0.8	74	0.0
November 29, 2009	Goderich	+6.9	-	88	6.1
November 30, 2009	Goderich	+4.0	+0.8	60	0.7
March 1, 2010	Goderich	+0.9	–9.8	84	0.0
March 2, 2010	Goderich	–1.0	–9.4	85	0.0
March 3, 2010	Goderich	+0.8	–8.2	75	0.0
March 4, 2010	Goderich	–0.2	–9.1	70	0.0
March 5, 2010	Goderich	–0.6	–11.6	83	0.0
March 6, 2010	Goderich	+2.1	–13.8	79	0.3
March 7, 2010	Goderich	+4.0	–1.5	75	0.0
March 8, 2010	Goderich	+5.7	–6.5	71	0.0
March 9, 2010	Goderich	+8.3	–4.5	77	0.0
March 10, 2010	Goderich	+8.8	–0.8	67	0.0
March 11, 2010	Goderich	+12.3	+3.8	64	0.0
March 12, 2010	Goderich	+12.1	+5.5	78	0.0
March 13, 2010	Goderich	+10.4	+3.8	84	2.2
March 14, 2010	Goderich	+7.3	+3.7	79	0.0
March 15, 2010	Goderich	+11.9	+0.3	75	0.0
March 16, 2010	Goderich	+10.3	–2.3	69	0.0
March 17, 2010	Goderich	+11.0	–2.9	57	0.0
March 18, 2010	Goderich	+14.2	+0.5	55	0.0
March 19, 2010	Goderich	+14.9	0.0	74	0.0
March 20, 2010	Goderich	+2.3	–1.2	77	0.0
March 21, 2010	Goderich	+4.9	–1.0	72	0.0
March 22, 2010	Goderich	+11.4	–1.2	53	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 23, 2010	Goderich	+7.2	–3.5	72	0.0
March 24, 2010	Goderich	+7.5	–3.4	70	0.0
March 25, 2010	Goderich	+7.8	–3.2	61	0.0
March 26, 2010	Goderich	–1.4	–6.5	58	0.0
March 27, 2010	Goderich	+7.5	–5.6	53	0.0
March 28, 2010	Goderich	+9.6	+2.5	69	6.3
March 29, 2010	Goderich	+5.2	–3.0	90	0.0
March 30, 2010	Goderich	+7.3	–4.7	80	0.0
March 31, 2010	Goderich	+18.8	+0.8	61	0.0
April 1, 2010	Goderich	+22.9	+12.4	48	0.0
April 2, 2010	Goderich	+24.6	+16.6	44	0.0
April 3, 2010	Goderich	+25.5	+7.2	49	0.0
April 4, 2010	Goderich	+17.9	+6.2	55	0.0
April 5, 2010	Goderich	+17.1	+9.0	60	0.0
April 6, 2010	Goderich	+15.6	+9.2	83	11.3
April 7, 2010	Goderich	+12.8	+4.4	95	3.5
April 8, 2010	Goderich	+14.2	+1.2	91	4.6
April 9, 2010	Goderich	+3.0	+0.3	71	0.0
April 10, 2010	Goderich	+13.2	+0.2	70	0.3
April 11, 2010	Goderich	+14.3	–0.7	65	0.0
April 12, 2010	Goderich	+10.5	–1.8	67	0.0
April 13, 2010	Goderich	+11.0	+1.4	60	0.0
April 14, 2010	Goderich	+15.7	+0.6	51	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
April 15, 2010	Goderich	+24.7	+7.8	46	0.0
April 16, 2010	Goderich	+18.5	+3.1	62	0.0
April 17, 2010	Goderich	+3.9	+0.7	82	0.0
April 18, 2010	Goderich	+7.6	+0.3	82	0.0
April 19, 2010	Goderich	+8.8	–1.7	79	0.0
April 20, 2010	Goderich	+11.3	–1.7	68	0.0
April 21, 2010	Goderich	+10.8	+0.4	77	0.0
April 22, 2010	Goderich	+5.4	–2.5	74	0.0
April 23, 2010	Goderich	+11.7	–4.3	64	0.0
April 24, 2010	Goderich	+19.1	+3.7	37	0.0
April 25, 2010	Goderich	+13.1	+7.8	72	9.0
April 26, 2010	Goderich	+16.6	+2.7	58	0.0
April 27, 2010	Goderich	+6.3	+1.3	62	0.0
April 28, 2010	Goderich	+9.1	–0.4	66	0.0
April 29, 2010	Goderich	+15.1	–2.1	60	0.0
April 30, 2010	Goderich	+24.0	+12.8	49	0.0
May 1, 2010	Goderich	+22.8	+14.4	69	6.7
May 2, 2010	Goderich	+22.0	+10.5	87	11.1
May 3, 2010	Goderich	+20.5	+9.8	81	2.6
May 4, 2010	Goderich	+19.3	+9.4	67	0.0
May 5, 2010	Goderich	+21.4	+12.0	68	6.1
May 6, 2010	Goderich	+12.1	+5.8	73	0.0
May 7, 2010	Goderich	+11.2	+3.8	84	19.9
May 8, 2010	Goderich	+8.5	+1.3	86	2.7
May 9, 2010	Goderich	+5.2	+1.6	67	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
May 10, 2010	Goderich	+7.2	+2.2	73	0.0
May 11, 2010	Goderich	+9.7	+3.1	72	3.5
May 12, 2010	Goderich	+14.3	+4.2	75	0.0
May 13, 2010	Goderich	+12.4	+5.2	86	15.6
May 14, 2010	Goderich	+15.7	+6.6	81	0.0
May 15, 2010	Goderich	+11.6	+3.8	83	0.0
May 16, 2010	Goderich	+12.0	+1.9	84	0.0
May 17, 2010	Goderich	+19.7	+6.3	53	0.0
May 18, 2010	Goderich	+21.0	+7.3	60	0.0
May 19, 2010	Goderich	+19.0	+5.9	68	0.0
May 20, 2010	Goderich	+22.3	+7.4	65	0.0
May 21, 2010	Goderich	+26.5	+9.4	56	0.0
May 22, 2010	Goderich	+19.9	+15.4	81	0.6
May 23, 2010	Goderich	+24.9	+14.7	83	0.0
May 24, 2010	Goderich	+26.4	+15.1	72	0.0
May 25, 2010	Goderich	+26.4	+16.4	71	0.0
May 26, 2010	Goderich	+26.1	+15.8	74	0.0
May 27, 2010	Goderich	+18.5	+13.5	80	0.0
May 28, 2010	Goderich	+22.8	+12.4	72	0.0
May 29, 2010	Goderich	+23.8	+11.4	68	0.0
May 30, 2010	Goderich	+28.0	+12.2	54	0.0
May 31, 2010	Goderich	+28.8	+16.1	60	2.4
June 1, 2010	Goderich	+21.2	+12.8	72	0.4
June 2, 2010	Goderich	+24.0	+13.4	79	20.0
June 3, 2010	Goderich	+20.1	+11.8	95	8.8

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
June 4, 2010	Goderich	+21.1	+10.2	88	8.7
June 5, 2010	Goderich	+22.0	+14.4	88	20.2
June 6, 2010	Goderich	+15.8	+8.4	87	11.0
June 7, 2010	Goderich	+14.1	+5.7	81	0.0
June 8, 2010	Goderich	+17.6	+4.8	74	0.0
June 9, 2010	Goderich	+19.9	+10.9	86	6.3
June 10, 2010	Goderich	+16.0	+12.2	85	0.0
June 11, 2010	Goderich	+25.4	+12.5	70	0.0
June 12, 2010	Goderich	+22.2	+12.7	90	0.0
June 13, 2010	Goderich	+19.2	+13.3	98	0.4
June 14, 2010	Goderich	+20.0	+14.0	84	0.0
June 15, 2010	Goderich	+25.1	+15.2	58	0.0
June 16, 2010	Goderich	+20.6	+12.8	79	2.7
June 17, 2010	Goderich	+18.8	+11.5	81	0.0
June 18, 2010	Goderich	+27.1	+13.8	66	0.0
June 19, 2010	Goderich	+27.9	+16.3	71	0.0
June 20, 2010	Goderich	+18.9	+14.5	87	0.0
June 21, 2010	Goderich	+25.5	+12.5	75	0.0
June 22, 2010	Goderich	+22.8	+17.5	81	15.3
June 23, 2010	Goderich	+26.5	+15.9	84	0.7
June 24, 2010	Goderich	+20.3	+15.2	92	54.4
June 25, 2010	Goderich	+22.8	+14.5	73	0.6
June 26, 2010	Goderich	+24.6	+18.1	76	0.6
June 27, 2010	Goderich	+24.7	+17.4	97	32.6
June 28, 2010	Goderich	+24.9	+14.6	89	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
June 29, 2010	Goderich	+16.1	+8.9	68	0.0
June 30, 2010	Goderich	+17.1	+8.8	69	0.0
July 1, 2010	Goderich	+17.4	+9.1	80	0.0
July 2, 2010	Goderich	+23.4	+7.8	63	0.0
July 3, 2010	Goderich	+26.4	+15.2	60	0.0
July 4, 2010	Goderich	+29.6	+18.2	58	0.0
July 5, 2010	Goderich	+31.4	+23.9	68	0.0
July 6, 2010	Goderich	+30.5	+23.3	61	0.0
July 7, 2010	Goderich	+31.1	+22.1	70	0.0
July 8, 2010	Goderich	+28.7	+21.9	80	0.3
July 9, 2010	Goderich	+23.2	+15.8	88	7.4
July 10, 2010	Goderich	+23.8	+13.9	76	0.0
July 11, 2010	Goderich	+27.6	+16.9	76	0.7
July 12, 2010	Goderich	+24.5	+17.7	86	17.5
July 13, 2010	Goderich	+22.6	+17.7	92	0.0
July 14, 2010	Goderich	+25.3	+16.9	88	0.0
July 15, 2010	Goderich	+29.7	+20.0	80	8.1
July 16, 2010	Goderich	+26.3	+20.2	79	0.0
July 17, 2010	Goderich	+26.3	+14.8	79	0.0
July 18, 2010	Goderich	+26.0	+14.0	81	14.9
July 19, 2010	Goderich	+20.9	+17.0	90	0.0
July 20, 2010	Goderich	+23.9	+16.9	78	0.0
July 21, 2010	Goderich	+23.8	+16.7	85	0.0
July 22, 2010	Goderich	+23.6	+15.0	86	16.0
July 23, 2010	Goderich	+26.8	+17.2	90	16.9

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
July 24, 2010	Goderich	+24.0	+18.3	94	2.9
July 25, 2010	Goderich	+22.4	+14.1	86	0.0
July 26, 2010	Goderich	+24.3	+13.7	82	0.0
July 27, 2010	Goderich	+26.5	+14.4	77	0.0
July 28, 2010	Goderich	+26.6	+18.7	82	3.3
July 29, 2010	Goderich	+21.6	+13.3	74	0.0
July 30, 2010	Goderich	+21.7	+13.4	79	0.0
July 31, 2010	Goderich	+20.8	+14.6	87	1.9
August 1, 2010	Goderich	+24.8	+17.9	79	0.5
August 2, 2010	Goderich	+27.2	+18.5	77	0.0
August 3, 2010	Goderich	+27.9	+21.0	78	0.0
August 4, 2010	Goderich	+27.1	+20.0	87	0.0
August 5, 2010	Goderich	+25.7	+19.3	83	0.0
August 6, 2010	Goderich	+21.0	+9.9	66	0.0
August 7, 2010	Goderich	+24.2	+9.6	71	0.0
August 8, 2010	Goderich	+25.8	+20.4	81	3.5
August 9, 2010	Goderich	+25.5	+19.6	84	0.6
August 10, 2010	Goderich	+24.2	+18.1	92	0.0
August 11, 2010	Goderich	+26.4	+16.1	92	0.0
August 12, 2010	Goderich	+28.1	+19.2	82	0.0
August 13, 2010	Goderich	+28.7	+18.6	76	0.0
August 14, 2010	Goderich	+29.1	+20.6	82	0.0
August 15, 2010	Goderich	+28.7	+21.5	82	8.2
August 16, 2010	Goderich	+23.8	+20.3	63	0.0
August 17, 2010	Goderich	+23.7	+15.0	65	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
August 18, 2010	Goderich	+23.8	+14.4	67	0.0
August 19, 2010	Goderich	+24.6	+14.5	80	0.0
August 20, 2010	Goderich	+25.8	+12.8	64	0.0
August 21, 2010	Goderich	+26.2	+18.2	74	0.0
August 22, 2010	Goderich	+20.8	+17.1	93	0.0
August 23, 2010	Goderich	+24.3	+17.9	85	0.0
August 24, 2010	Goderich	+23.4	+16.5	68	0.0
August 25, 2010	Goderich	+21.4	+16.3	76	0.0
August 26, 2010	Goderich	+18.9	+8.2	60	0.0
August 27, 2010	Goderich	+22.7	+7.9	61	0.0
August 28, 2010	Goderich	+27.5	+16.5	49	0.0
August 29, 2010	Goderich	+30.4	+16.9	37	0.0
August 30, 2010	Goderich	+31.0	+21.8	61	0.0
August 31, 2010	Goderich	+31.1	+22.0	54	0.0
October 1, 2010	Goderich	+15.4	+4.9	61	0.0
October 2, 2010	Goderich	+11.7	+5.2	71	4.5
October 3, 2010	Goderich	+13.0	+3.0	50	0.0
October 4, 2010	Goderich	+13.6	+1.7	48	0.0
October 5, 2010	Goderich	+15.1	+6.4	61	0.0
October 6, 2010	Goderich	+16.7	+5.8	75	0.0
October 7, 2010	Goderich	+16.3	+6.2	65	0.0
October 8, 2010	Goderich	+21.0	+10.4	64	0.0
October 9, 2010	Goderich	+18.6	+6.6	38	0.0
October 10, 2010	Goderich	+16.6	+4.4	67	0.0
October 11, 2010	Goderich	+17.1	+5.0	81	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
October 12, 2010	Goderich	+13.1	+3.1	59	0.0
October 13, 2010	Goderich	+14.1	+3.9	81	7.8
October 14, 2010	Goderich	+14.6	+7.6	66	5.8
October 15, 2010	Goderich	+14.1	+5.5	64	0.4
October 16, 2010	Goderich	+14.5	+1.4	63	0.0
October 17, 2010	Goderich	+14.2	+3.1	55	0.0
October 18, 2010	Goderich	+12.3	+1.2	54	0.0
October 19, 2010	Goderich	+13.0	+5.4	45	0.0
October 20, 2010	Goderich	+15.9	+7.4	57	0.0
October 21, 2010	Goderich	+9.4	+3.1	72	1.1
October 22, 2010	Goderich	+12.1	+3.1	60	0.8
October 23, 2010	Goderich	+15.0	+5.2	74	2.7
October 24, 2010	Goderich	+19.1	+11.6	52	9.4
October 25, 2010	Goderich	+18.3	+15.0	76	1.2
October 26, 2010	Goderich	+20.6	+13.0	71	1.7
October 27, 2010	Goderich	+17.1	+11.6	54	0.0
October 28, 2010	Goderich	+13.1	+5.9	68	0.0
October 29, 2010	Goderich	+9.1	+4.3	65	0.0
October 30, 2010	Goderich	+12.1	+5.9	53	0.0
October 31, 2010	Goderich	+6.7	+0.6	57	0.0
November 1, 2010	Goderich	+6.3	–2.5	64	0.0
November 2, 2010	Goderich	+7.3	–4.3	56	0.0
November 3, 2010	Goderich	+7.7	–3.4	63	0.2
November 4, 2010	Goderich	+8.5	+3.6	84	4.4
November 5, 2010	Goderich	+3.8	0.0	94	5.8

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
November 6, 2010	Goderich	+5.8	–0.1	70	0.3
November 7, 2010	Goderich	+8.3	0.2	65	0.0
November 8, 2010	Goderich	+10.4	–1.8	70	0.0
November 9, 2010	Goderich	+12.5	–2.5	63	0.0
November 10, 2010	Goderich	+11.4	+1.2	73	0.0
November 11, 2010	Goderich	+13.7	–2.0	54	0.0
November 12, 2010	Goderich	+12.3	+0.7	81	0.0
November 13, 2010	Goderich	+14.9	–0.7	62	0.2
November 14, 2010	Goderich	+11.2	+3.6	75	6.2
November 15, 2010	Goderich	+8.6	+0.4	69	0.0
November 16, 2010	Goderich	+10.6	+0.1	56	9.4
November 17, 2010	Goderich	+8.7	+4.9	79	13.0
November 18, 2010	Goderich	+7.0	+2.3	85	2.9
November 19, 2010	Goderich	+6.6	+1.1	61	0.0
November 20, 2010	Goderich	+6.6	–0.7	63	0.0
November 21, 2010	Goderich	+14.5	–2.0	66	0.0
November 22, 2010	Goderich	+16.7	+11.5	92	12.9
November 23, 2010	Goderich	+13.7	+0.8	66	1.9
November 24, 2010	Goderich	+2.5	–1.8	60	0.0
November 25, 2010	Goderich	+7.4	+0.4	83	11.9
November 26, 2010	Goderich	+1.3	–2.8	66	0.0
November 27, 2010	Goderich	+3.4	–1.0	71	1.8
November 28, 2010	Goderich	+2.4	–1.4	72	0.0
November 29, 2010	Goderich	+6.9	–1.0	70	0.0
November 30, 2010	Goderich	+12.6	+3.3	86	18.2

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 1, 2011	Goderich	+0.2	–4.4	74	0.0
March 2, 2011	Goderich	+0.9	–8.4	72	0.0
March 3, 2011	Goderich	–3.4	–12.3	69	0.0
March 4, 2011	Goderich	+5.2	–3.5	70	10.6
March 5, 2011	Goderich	+4.9	–5.4	96	25.3
March 6, 2011	Goderich	–3.8	–9.2	78	0.0
March 7, 2011	Goderich	–3.2	–11.0	71	0.0
March 8, 2011	Goderich	+3.2	–5.7	62	0.0
March 9, 2011	Goderich	+4.3	–0.8	82	10.0
March 10, 2011	Goderich	+5.2	–0.1	98	5.9
March 11, 2011	Goderich	+0.4	–1.2	100	3.8
March 12, 2011	Goderich	+2.7	–3.3	95	0.7
March 13, 2011	Goderich	0.0	–3.4	92	0.3
March 14, 2011	Goderich	–0.7	–10.9	81	0.0
March 15, 2011	Goderich	+5.5	–8.4	72	1.1
March 16, 2011	Goderich	+7.2	+0.4	84	2.3
March 17, 2011	Goderich	+15.1	+4.9	70	0.0
March 18, 2011	Goderich	+10.4	–0.7	88	0.0
March 19, 2011	Goderich	+0.3	–4.5	80	0.0
March 20, 2011	Goderich	+4.1	–4.9	74	0.8
March 21, 2011	Goderich	+6.0	+1.0	94	1.7
March 22, 2011	Goderich	+4.0	–1.9	81	0.6
March 23, 2011	Goderich	–1.9	–5.0	84	8.6
March 24, 2011	Goderich	–4.7	–19.1	72	0.0
March 25, 2011	Goderich	–4.3	–19.5	67	0.3

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 26, 2011	Goderich	–5.2	–19.6	75	0.0
March 27, 2011	Goderich	–3.5	–18.0	68	0.0
March 28, 2011	Goderich	–1.6	–4.7	58	0.0
March 29, 2011	Goderich	0.0	–7.0	76	0.0
March 30, 2011	Goderich	+2.4	–8.4	86	0.0
March 31, 2011	Goderich	+4.0	–0.8	79	0.0
April 1, 2011	Goderich	+4.5	–2.2	82	0.0
April 2, 2011	Goderich	+5.6	–4.1	77	0.0
April 3, 2011	Goderich	+7.0	–1.4	83	10.7
April 4, 2011	Goderich	+15.7	+1.9	89	12.0
April 5, 2011	Goderich	+2.8	+0.1	76	0.0
April 6, 2011	Goderich	+3.8	–1.1	85	0.0
April 7, 2011	Goderich	+7.1	–2.5	79	0.0
April 8, 2011	Pinery	+12.8	+0.6	68	0.0
April 9, 2011	Pinery	+11.8	+0.2	74	0.0
April 10, 2011	Pinery	+23.2	+6.0	75	9.4
April 11, 2011	Pinery	+21.4	+3.0	72	0.0
April 12, 2011	Pinery	+12.9	+1.2	75	0.0
April 13, 2011	Pinery	+9.3	–0.6	72	0.0
April 14, 2011	Pinery	+10.3	+0.2	76	0.0
April 15, 2011	Pinery	+9.9	–0.7	42	0.0
April 16, 2011	Pinery	+12.3	+1.6	79	5.1
April 17, 2011	Pinery	+3.1	0.0	70	0.0
April 18, 2011	Pinery	+1.5	–2.2	75	0.0
April 19, 2011	Pinery	+7.5	–1.9	67	7.3

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
April 20, 2011	Pinery	+3.8	+1.1	86	15.2
April 21, 2011	Pinery	+4.4	–0.7	75	0.0
April 22, 2011	Pinery	+11.6	+1.3	73	3.4
April 23, 2011	Pinery	+15.5	+4.8	72	8.0
April 24, 2011	Pinery	+7.9	+2.7	80	0.0
April 25, 2011	Pinery	+13.7	+3.7	79	0.0
April 26, 2011	Pinery	+20.9	+7.6	80	2.7
April 27, 2011	Pinery	+20.3	+10.8	87	7.9
April 28, 2011	Pinery	+16.4	+3.5	-	11.4
April 29, 2011	Pinery	+7.1	–0.4	76	0.0
April 30, 2011	Pinery	+17.0	–1.2	70	0.3
May 1, 2011	Pinery	+16.3	+8.3	75	0.0
May 2, 2011	Pinery	+10.5	+5.8	69	0.0
May 3, 2011	Pinery	+7.9	+3.4	87	0.9
May 4, 2011	Pinery	+7.3	+3.3	79	0.0
May 5, 2011	Pinery	+14.8	+4.2	57	0.0
May 6, 2011	Pinery	+14.1	+5.5	74	1.0
May 7, 2011	Pinery	+10.8	+4.6	82	0.0
May 8, 2011	Pinery	+15.3	+4.5	74	0.0
May 9, 2011	Pinery	+15.5	+3.1	67	0.0
May 10, 2011	Pinery	+17.8	+10.6	51	0.0
May 11, 2011	Pinery	+23.4	+11.0	47	0.0
May 12, 2011	Pinery	+26.3	+10.2	59	0.0
May 13, 2011	Pinery	+23.3	+12.0	72	37.2
May 14, 2011	Pinery	+17.5	+10.9	93	30.8

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
May 15, 2011	Pinery	+12.3	+6.5	87	1.5
May 16, 2011	Pinery	+9.8	+4.0	83	0.3
May 17, 2011	Pinery	+16.6	+5.4	85	0.9
May 18, 2011	Pinery	+20.3	+13.0	82	19.8
May 19, 2011	Pinery	+17.6	+11.1	86	12.7
May 20, 2011	Pinery	+17.5	+8.7	93	0.0
May 21, 2011	Pinery	+20.3	+8.2	81	0.0
May 22, 2011	Pinery	+26.1	+14.9	74	4.8
May 23, 2011	Pinery	+22.0	+12.5	74	6.8
May 24, 2011	Pinery	+14.2	+6.1	77	0.0
May 25, 2011	Pinery	+17.2	+5.1	86	9.3
May 26, 2011	Pinery	+17.1	+7.9	93	4.9
May 27, 2011	Pinery	+15.3	+8.2	92	0.0
May 28, 2011	Pinery	+18.1	+10.7	86	0.0
May 29, 2011	Pinery	+20.1	+13.9	88	3.9
May 30, 2011	Pinery	+27.9	+11.8	-	0.0
May 31, 2011	Pinery	+29.7	+17.4	50	0.0
June 1, 2011	Pinery	+19.3	+10.6	55	0.0
June 2, 2011	Pinery	+12.4	+5.1	69	0.0
June 3, 2011	Pinery	+20.3	+4.5	65	0.0
June 4, 2011	Pinery	+24.4	+11.3	67	0.0
June 5, 2011	Pinery	+21.2	+10.5	78	0.0
June 6, 2011	Pinery	+26.7	+9.6	61	0.0
June 7, 2011	Pinery	+28.3	+17.4	74	43.6
June 8, 2011	Pinery	+29.7	+20.5	-	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
June 9, 2011	Pinery	+24.9	+8.4	84	12.3
June 10, 2011	Pinery	+19.1	+7.2	80	0.0
June 11, 2011	Pinery	+19.8	+11.7	82	0.0
June 12, 2011	Pinery	+13.7	+9.4	78	0.0
June 13, 2011	Pinery	+15.9	+8.2	77	0.0
June 14, 2011	Pinery	+22.2	+8.0	81	0.0
June 15, 2011	Pinery	+24.2	+8.6	66	0.0
June 16, 2011	Pinery	+21.6	+13.3	79	0.8
June 17, 2011	Pinery	+20.2	+12.6	89	0.0
June 18, 2011	Pinery	+25.0	+12.4	81	0.0
June 19, 2011	Pinery	+24.5	+14.2	55	0.0
June 20, 2011	Pinery	+25.6	+14.2	61	0.0
June 21, 2011	Pinery	+27.8	+17.5	76	0.7
June 22, 2011	Pinery	+26.7	+16.6	86	22.3
June 23, 2011	Pinery	+20.9	+16.5	90	1.0
June 24, 2011	Pinery	+17.1	+14.2	88	3.0
June 25, 2011	Pinery	+17.3	+12.9	85	0.7
June 26, 2011	Pinery	+19.0	+10.3	84	0.0
June 27, 2011	Pinery	+23.3	+11.8	76	3.3
June 28, 2011	Pinery	+21.0	+13.3	79	0.0
June 29, 2011	Pinery	+16.7	+8.5	80	0.0
June 30, 2011	Pinery	+19.9	+7.6	71	0.0
July 1, 2011	Pinery	+26.8	+8.0	62	0.0
July 2, 2011	Pinery	+29.5	+19.0	74	0.0
July 3, 2011	Pinery	+22.4	+13.0	80	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
July 4, 2011	Pinery	+22.2	+11.2	73	0.0
July 5, 2011	Pinery	+27.1	+11.9	69	0.0
July 6, 2011	Goderich	+23.9	+14.5	81	0.9
July 7, 2011	Goderich	+21.1	+11.8	84	0.0
July 8, 2011	Goderich	+23.9	+11.3	79	0.0
July 9, 2011	Goderich	+25.4	+11.9	77	0.0
July 10, 2011	Goderich	+28.7	+19.8	65	0.0
July 11, 2011	Goderich	+28.6	+20.5	70	0.0
July 12, 2011	Goderich	+26.1	+17.4	78	0.0
July 13, 2011	Goderich	+21.1	+12.2	76	0.0
July 14, 2011	Goderich	+24.1	+10.8	66	0.0
July 15, 2011	Pinery	+27.7	+13.4	62	0.0
July 16, 2011	Pinery	+29.3	+15.9	68	0.0
July 17, 2011	Pinery	+31.5	+21.2	62	0.0
July 18, 2011	Pinery	+27.6	+19.6	75	3.2
July 19, 2011	Pinery	+27.5	+18.7	87	0.0
July 20, 2011	Pinery	+30.3	+19.4	83	0.0
July 21, 2011	Pinery	+32.5	+19.2	65	0.0
July 22, 2011	Pinery	+27.5	+16.6	80	0.3
July 23, 2011	Pinery	+28.5	+21.6	84	0.0
July 24, 2011	Pinery	+27.1	+19.9	89	0.0
July 25, 2011	Pinery	+23.5	+19.6	84	0.0
July 26, 2011	Pinery	+22.8	+12.5	72	0.0
July 27, 2011	Pinery	+23.6	+11.1	76	0.0
July 28, 2011	Pinery	+25.7	+19.9	85	19.9

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
July 29, 2011	Pinery	+25.8	+16.9	86	10.0
July 30, 2011	Pinery	+27.0	+14.5	75	0.0
July 31, 2011	Pinery	+28.6	+21.0	74	0.3
August 1, 2011	Pinery	+25.1	+15.9	74	0.0
August 2, 2011	Pinery	+25.1	+15.1	82	0.4
August 3, 2011	Pinery	+23.7	+17.8	91	0.3
August 4, 2011	Pinery	+25.5	+15.5	80	0.0
August 5, 2011	Pinery	+26.7	+15.6	79	0.0
August 6, 2011	Pinery	+27.7	+20.3	82	0.0
August 7, 2011	Pinery	+25.6	+18.8	87	15.8
August 8, 2011	Pinery	+24.6	+16.9	79	0.0
August 9, 2011	Goderich	+23.7	+18.3	69	1.7
August 10, 2011	Goderich	+21.2	+16.9	51	7.1
August 11, 2011	Goderich	+23.2	+15.1	42	0.0
August 12, 2011	Goderich	+24.9	+18.2	43	0.0
August 13, 2011	Goderich	+25.8	+17.6	44	0.0
August 14, 2011	Goderich	+24.5	+16.1	85	18.0
August 15, 2011	Goderich	+22.9	+11.5	69	0.0
August 16, 2011	Goderich	+23.2	+11.4	53	0.0
August 17, 2011	Goderich	+25.7	+13.7	47	0.0
August 18, 2011	Goderich	+25.2	+14.5	51	0.0
August 19, 2011	Goderich	+25.6	+13.0	43	0.0
August 20, 2011	Goderich	+24.0	+17.5	51	29.4
August 21, 2011	Goderich	+21.9	+14.4	56	15.5
August 22, 2011	Goderich	+20.5	+15.9	48	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
August 23, 2011	Goderich	+23.9	+11.6	43	0.0
August 24, 2011	Goderich	+26.1	+16.9	77	20.9
August 25, 2011	Goderich	+20.3	+11.9	88	0.0
August 26, 2011	Goderich	+22.0	+10.2	83	0.0
August 27, 2011	Goderich	+23.3	+15.5	75	0.0
August 28, 2011	Goderich	+22.0	+10.9	52	0.0
August 29, 2011	Goderich	+21.8	+10.9	72	0.2
August 30, 2011	Goderich	+24.0	+14.9	62	0.0
August 31, 2011	Goderich	+24.8	+15.6	52	0.0
October 1, 2011	Goderich	+10.1	+4.8	59	0.0
October 2, 2011	Goderich	+11.4	+3.5	57	1.1
October 3, 2011	Goderich	+12.6	+8.8	95	5.3
October 4, 2011	Goderich	+15.8	+4.7	76	0.2
October 5, 2011	Goderich	+18.9	+5.7	78	0.2
October 6, 2011	Goderich	+20.5	+9.6	48	0.0
October 7, 2011	Goderich	+23.1	+9.6	53	0.0
October 8, 2011	Goderich	+23.5	+9.6	51	0.0
October 9, 2011	Goderich	+24.8	+13.2	53	0.0
October 10, 2011	Goderich	+24.2	+8.8	47	0.4
October 11, 2011	Goderich	+26.6	+10.0	40	0.0
October 12, 2011	Goderich	+19.0	+13.3	77	13.4
October 13, 2011	Goderich	+16.9	+13.2	84	4.7
October 14, 2011	Goderich	+15.0	+10.4	82	11.6
October 15, 2011	Goderich	+11.4	+7.6	87	5.2
October 16, 2011	Goderich	+13.2	+10.6	71	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
October 17, 2011	Goderich	+12.8	+9.4	59	0.0
October 18, 2011	Goderich	+12.3	+7.0	65	0.0
October 19, 2011	Goderich	+10.5	+6.9	81	30.1
October 20, 2011	Goderich	+10.3	+7.7	95	14.2
October 21, 2011	Goderich	+8.6	+6.8	84	1.3
October 22, 2011	Goderich	+9.1	+4.9	66	0.0
October 23, 2011	Goderich	+16.6	+2.7	48	0.0
October 24, 2011	Goderich	+13.1	+8.5	65	2.6
October 25, 2011	Goderich	+10.1	+3.8	84	11.1
October 26, 2011	Goderich	+8.3	+3.7	87	0.0
October 27, 2011	Goderich	+5.9	+0.1	64	0.0
October 28, 2011	Goderich	+8.9	+1.3	67	0.8
October 29, 2011	Goderich	+9.9	+2.0	52	0.0
October 30, 2011	Goderich	+9.5	+2.4	58	2.3
October 31, 2011	Goderich	+13.2	+5.2	51	0.6
November 1, 2011	Goderich	+12.5	+5.1	62	0.0
November 2, 2011	Goderich	+17.0	+7.9	43	0.0
November 3, 2011	Goderich	+12.4	+2.1	75	0.0
November 4, 2011	Goderich	+9.2	–0.8	58	0.0
November 5, 2011	Goderich	+10.7	+0.1	48	0.0
November 6, 2011	Goderich	+15.7	+4.0	51	0.0
November 7, 2011	Goderich	+13.6	+9.9	78	0.0
November 8, 2011	Goderich	+16.0	+8.9	95	0.0
November 9, 2011	Goderich	+16.4	+6.3	75	1.8
November 10, 2011	Goderich	+8.1	+1.9	62	0.6

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
November 11, 2011	Goderich	+5.7	+1.5	91	1.5
November 12, 2011	Goderich	+11.3	+2.1	70	0.2
November 13, 2011	Goderich	+15.0	+10.6	64	1.2
November 14, 2011	Goderich	+12.1	+3.7	72	0.0
November 15, 2011	Goderich	+11.9	+5.7	58	0.2
November 16, 2011	Goderich	+10.1	+2.4	59	0.0
November 17, 2011	Goderich	+2.9	–0.2	60	0.0
November 18, 2011	Goderich	+6.5	+0.4	57	0.0
November 19, 2011	Goderich	+10.9	+5.7	61	0.0
November 20, 2011	Goderich	+11.5	+1.2	79	0.0
November 21, 2011	Goderich	+2.9	–4.5	64	0.0
November 22, 2011	Goderich	+2.2	–3.5	65	2.8
November 23, 2011	Goderich	+6.4	–0.4	74	0.0
November 24, 2011	Goderich	+6.4	+3.9	89	0.0
November 25, 2011	Goderich	+12.3	+4.9	66	0.0
November 26, 2011	Goderich	+14.8	+9.3	62	0.0
November 27, 2011	Goderich	+13.8	+2.9	97	24.8
November 28, 2011	Goderich	+3.7	+2.0	78	2.0
November 29, 2011	Goderich	+3.4	+0.3	93	35.0
November 30, 2011	Goderich	+4.9	–0.1	65	8.8
March 1, 2012	Goderich	+1.9	–0.1	97	4.0
March 2, 2012	Goderich	+9.1	+0.3	80	3.8
March 3, 2012	Goderich	+4.9	–3.7	79	1.5
March 4, 2012	Goderich	–3.5	–12.4	77	0.3
March 5, 2012	Goderich	–4.8	–16.1	70	0.5

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 6, 2012	Goderich	+9.1	–7.0	54	0.0
March 7, 2012	Goderich	+15.1	+7.7	48	0.0
March 8, 2012	Goderich	+13.7	–0.9	92	0.3
March 9, 2012	Goderich	+0.7	–5.3	57	1.0
March 10, 2012	Goderich	+4.9	–8.4	55	0.0
March 11, 2012	Goderich	+14.9	+3.8	43	0.0
March 12, 2012	Goderich	+13.6	+6.0	100	11.2
March 13, 2012	Goderich	+12.0	–0.4	77	0.0
March 14, 2012	Goderich	+18.4	–1.4	31	0.3
March 15, 2012	Goderich	+18.7	+7.6	68	1.7
March 16, 2012	Goderich	+11.6	+4.0	100	0.0
March 17, 2012	Goderich	+21.6	+10.8	62	0.0
March 18, 2012	Goderich	+21.5	+11.3	73	0.0
March 19, 2012	Goderich	+20.8	+10.9	76	0.0
March 20, 2012	Goderich	+25.0	+14.7	58	0.0
March 21, 2012	Goderich	+25.1	+15.4	49	0.0
March 22, 2012	Goderich	+25.0	+8.1	70	0.0
March 23, 2012	Goderich	+20.8	+10.2	48	0.0
March 24, 2012	Goderich	+15.2	+6.7	49	0.0
March 25, 2012	Goderich	+10.4	+2.1	69	0.0
March 26, 2012	Goderich	+3.0	-5.8	60	0.0
March 27, 2012	Goderich	+10.0	-6.5	26	0.0
March 28, 2012	Goderich	+10.9	+3.4	79	0.0
March 29, 2012	Goderich	+3.5	–4.4	68	0.0
March 30, 2012	Goderich	+4.9	–5.1	50	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
March 31, 2012	Goderich	+5.5	–1.5	71	0.0
April 1, 2012	Goderich	+5.5	+1.4	100	0.8
April 2, 2012	Goderich	+8.6	–2.1	77	0.0
April 3, 2012	Goderich	+12.8	–1.3	57	0.0
April 4, 2012	Goderich	+7.1	+1.3	75	0.0
April 5, 2012	Goderich	+3.8	–1.7	67	0.0
April 6, 2012	Goderich	+6.4	–4.6	67	0.0
April 7, 2012	Goderich	+10.4	–6.2	48	0.0
April 8, 2012	Goderich	+10.3	–0.7	82	0.7
April 9, 2012	Goderich	+9.7	+2.7	53	0.0
April 10, 2012	Goderich	+5.9	+1.0	66	0.0
April 11, 2012	Goderich	+5.3	–1.9	80	0.0
April 12, 2012	Goderich	+10.0	–3.9	70	0.0
April 13, 2012	Goderich	+13.8	–3.1	49	0.0
April 14, 2012	Goderich	+15.7	+6.5	68	0.0
April 15, 2012	Goderich	+20.6	+12.5	87	2.5
April 16, 2012	Goderich	+20.0	+3.8	65	0.0
April 17, 2012	Goderich	+4.9	–1.4	57	0.0
April 18, 2012	Goderich	+13.6	–2.8	51	0.0
April 19, 2012	Goderich	+11.3	+6.9	69	0.0
April 20, 2012	Goderich	+20.4	+2.1	71	6.4
April 21, 2012	Goderich	+8.0	+1.7	68	0.0
April 22, 2012	Goderich	+11.0	–0.3	38	0.0
April 23, 2012	Goderich	+7.4	+3.3	57	0.0
April 24, 2012	Goderich	+8.0	–1.3	60	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
April 25, 2012	Goderich	+12.8	–2.8	44	0.0
April 26, 2012	Goderich	+9.0	+2.5	90	0.4
April 27, 2012	Pinery	+11.5	+1.0	47	0.0
April 28, 2012	Pinery	+10.5	–2.5	48	0.0
April 29, 2012	Pinery	+17.0	–4.5	65	0.0
April 30, 2012	Pinery	+9.5	+2.5	78	5.7
May 1, 2012	Pinery	+13.0	+7.5	83	0.6
May 2, 2012	Pinery	+28.0	+7.5	72	0.0
May 3, 2012	Pinery	+32.5	+9.5	74	26.2
May 4, 2012	Pinery	+17.0	+9.5	95	0.0
May 5, 2012	Pinery	+16.5	+9.5	75	0.0
May 6, 2012	Pinery	+16.0	+6.5	53	0.0
May 7, 2012	Pinery	+16.0	+11.5	88	7.5
May 8, 2012	Pinery	+13.0	+9.5	90	0.0
May 9, 2012	Pinery	+16.0	+7.0	88	0.0
May 10, 2012	Pinery	+17.5	+7.5	77	0.0
May 11, 2012	Pinery	+28.5	+1.5	49	0.3
May 12, 2012	Pinery	+15.0	+10.5	53	1.5
May 13, 2012	Pinery	+18.5	+8.0	46	0.0
May 14, 2012	Pinery	+26.0	+3.5	36	0.0
May 15, 2012	Pinery	+29.5	+7.0	33	0.5
May 16, 2012	Pinery	+14.0	+10.5	70	2.9
May 17, 2012	Pinery	+18.0	+2.5	47	0.0
May 18, 2012	Pinery	+19.0	+5.0	37	0.0
May 19, 2012	Pinery	+29.0	+10.0	37	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
May 20, 2012	Pinery	+34.5	+13.5	27	0.0
May 21, 2012	Pinery	+28.0	+13.0	55	0.0
May 22, 2012	Pinery	+16.0	+10.5	77	0.0
May 23, 2012	Pinery	+21.0	+7.0	71	0.0
May 24, 2012	Pinery	+31.5	+14.5	46	0.0
May 25, 2012	Pinery	+29.5	+20.0	52	0.0
May 26, 2012	Pinery	+18.0	+14.5	66	0.0
May 27, 2012	Pinery	+20.0	+12.0	91	1.7
May 28, 2012	Pinery	+35.0	+16.5	59	0.0
May 29, 2012	Pinery	+26.5	+21.0	90	0.0
May 30, 2012	Pinery	+21.0	+11.0	56	0.0
May 31, 2012	Pinery	+16.0	+10.5	75	0.0
June 1, 2012	Pinery	+16.5	+11.0	94	10.4
June 2, 2012	Pinery	+19.5	+10.0	63	1.6
June 3, 2012	Pinery	+20.0	+11.5	78	0.0
June 4, 2012	Pinery	+16.0	+9.5	91	0.3
June 5, 2012	Pinery	+16.5	+9.5	81	0.0
June 6, 2012	Pinery	+20.5	+9.0	70	0.0
June 7, 2012	Pinery	+20.0	+11.0	70	0.0
June 8, 2012	Pinery	+18.5	+11.0	47	0.0
June 9, 2012	Pinery	+31.0	+16.5	59	15.1
June 10, 2012	Pinery	+30.5	+16.5	42	0.0
June 11, 2012	Pinery	+29.5	+18.5	54	1.3
June 12, 2012	Pinery	+21.5	+14.0	73	10.3
June 13, 2012	Pinery	+18.5	+11.5	71	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
June 14, 2012	Pinery	+26.0	+10.0	42	0.0
June 15, 2012	Pinery	+33.0	+14.0	45	0.0
June 16, 2012	Pinery	+31.0	+18.0	45	0.0
June 17, 2012	Pinery	+26.0	+19.5	76	2.8
June 18, 2012	Pinery	+27.5	+17.0	68	0.3
June 19, 2012	Pinery	+31.5	+23.0	59	0.4
June 20, 2012	Pinery	+34.5	+23.0	56	0.0
June 21, 2012	Pinery	+33.0	+22.0	51	1.7
June 22, 2012	Pinery	+27.5	+17.5	71	0.0
June 23, 2012	Pinery	+26.5	+13.0	73	0.0
June 24, 2012	Pinery	+27.5	+15.0	64	0.0
June 25, 2012	Pinery	+22.0	+16.5	67	0.0
June 26, 2012	Pinery	+24.0	+12.0	62	0.0
June 27, 2012	Pinery	+33.0	+13.5	41	0.0
June 28, 2012	Pinery	+34.0	+18.5	50	0.0
June 29, 2012	Pinery	+33.5	+20.0	58	0.0
June 30, 2012	Pinery	+35.5	+20.5	48	0.0
July 1, 2012	Pinery	+32.0	+19.5	58	0.0
July 2, 2012	Pinery	+33.5	+16.0	46	0.0
July 3, 2012	Pinery	+26.5	+20.5	85	4.6
July 4, 2012	Pinery	+34.0	+20.0	74	0.0
July 5, 2012	Pinery	+32.5	+21.5	70	0.0
July 6, 2012	Pinery	+31.0	+22.0	68	0.0
July 7, 2012	Pinery	+27.5	+23.0	74	0.0
July 8, 2012	Pinery	+26.0	+18.5	75	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
July 9, 2012	Pinery	+27.5	+13.5	59	0.0
July 10, 2012	Pinery	+26.5	+14.0	74	0.0
July 11, 2012	Pinery	+27.5	+14.0	56	0.0
July 12, 2012	Pinery	+28.5	+13.0	43	0.0
July 13, 2012	Pinery	+32.5	+17.0	58	0.0
July 14, 2012	Pinery	+30.5	+18.5	52	0.0
July 15, 2012	Pinery	+31.0	+21.0	70	0.3
July 16, 2012	Pinery	+31.5	+19.5	62	0.0
July 17, 2012	Pinery	+36.0	+24.5	53	0.3
July 18, 2012	Pinery	+28.0	+22.0	70	0.0
July 19, 2012	Pinery	+22.0	+18.0	86	0.0
July 20, 2012	Pinery	+26.5	+17.0	48	0.0
July 21, 2012	Pinery	+30.5	+12.5	48	0.0
July 22, 2012	Pinery	+32.0	+20.0	60	0.0
July 23, 2012	Pinery	+35.0	+23.0	50	1.0
July 24, 2012	Pinery	+25.0	+22.0	66	0.0
July 25, 2012	Pinery	+30.5	+13.5	48	17.8
July 26, 2012	Pinery	+27.0	+21.0	80	7.0
July 27, 2012	Pinery	+27.5	+18.5	74	3.5
July 28, 2012	Pinery	+26.5	+16.5	79	3.0
July 29, 2012	Pinery	+30.5	+14.0	73	0.3
July 30, 2012	Pinery	+30.5	+15.0	57	0.0
July 31, 2012	Pinery	+30.0	+20.5	69	0.0
August 1, 2012	Pinery	+28.5	+20.0	77	0.0
August 2, 2012	Pinery	+30.0	+16.0	73	0.0

Appendix 5 Continued.

Date	Location	Temperature (˚C)		Relative Humidity (%) 1:00 PM	Total Precipitation (mm)
		High	Low		
August 3, 2012	Pinery	+32.0	+21.0	57	0.0
August 4, 2012	Pinery	+35.5	+21.5	60	0.0
August 5, 2012	Pinery	+32.5	+23.0	84	0.0
August 6, 2012	Pinery	+24.0	+20.0	50	0.0
August 7, 2012	Pinery	+35.0	+15.0	47	0.7
August 8, 2012	Pinery	+27.0	+19.0	75	0.0
August 9, 2012	Pinery	+21.5	+18.5	96	2.9
August 10, 2012	Pinery	+26.0	+17.0	99	6.9
August 11, 2012	Pinery	+26.5	+15.0	80	1.9
August 12, 2012	Pinery	+29.0	+18.0	78	0.0
August 13, 2012	Pinery	+27.5	+17.0	67	12.3
August 14, 2012	Pinery	+27.0	+17.0	88	7.4
August 15, 2012	Pinery	+33.5	+14.0	70	0.0
August 16, 2012	Pinery	+28.5	+15.0	69	1.1
August 17, 2012	Pinery	+28.5	+19.0	70	0.0
August 18, 2012	Pinery	+29.0	+12.5	59	0.0
August 19, 2012	Pinery	+28.0	+12.0	61	0.0
August 20, 2012	Pinery	+24.0	+13.0	54	0.0
August 21, 2012	Pinery	+32.0	+9.5	59	0.0
August 22, 2012	Pinery	+30.5	+13.0	53	0.0
August 23, 2012	Pinery	+30.5	+15.0	52	0.0

Appendix 6 Oxygen isotope composition of precipitation at the Pinery, taken from an automated collector located at 43° 15' 1.7208"N, 81° 50' 56.7708"W.

Month, Year	δ^{18}O (VSMOW) (‰)
June, 2008	–6.0
July, 2008	–9.7
August, 2008	–14.3
September, 2008	–9.5
October, 2008	–7.9
November, 2008	–15.6
December, 2008	–13.3
January, 2009	–13.7
February, 2009	–19.3
March, 2009	–10.1
April, 2009	–10.4
May, 2009	–6.2
June, 2009	–6.9
July, 2009	–7.3
August, 2009	–8.8
September, 2009	–6.9
October, 2009	–10.7
November, 2009	–11.1
December, 2009	–11.0
January, 2010	n/a
February, 2010	n/a
March, 2010	–14.7
April, 2010	–11.2
May, 2010	–5.6
June, 2010	–6.1
July, 2010	–6.0
August, 2010	–6.3

Month, Year	δ^{18}O (VSMOW) (‰)
September, 2010	–5.9
October, 2010	–10.5
November, 2010	–11.2
December, 2010	–11.1
January, 2011	–15.8
February, 2011	–18.8
March, 2011	–12.9
April, 2011	–11.2
May, 2011	–7.3
June, 2011	–6.4
July, 2011	–6.6
August, 2011	–5.5
September, 2011	–5.7
October, 2011	–9.6
November, 2011	–13.1
December, 2011	–12.5
January, 2012	–13.6
February, 2012	–12.9
March, 2012	–13.3
April, 2012	–4.6
May, 2012	–4.1
June, 2012	–6.2
July, 2012	–4.1
August, 2012	–8.0
September, 2012	–7.6
October, 2012	–9.9
November, 2012	–10.7

Curriculum Vitae

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